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(54) **METHOD OF TREATING HEREDITARY ANGIOEDEMA USING PLASMA KALLIKREIN BINDING ANTIBODIES**

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(58) **Field of Classification Search**
None
See application file for complete search history.

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(57) **ABSTRACT**

Plasma kallikrein binding proteins and methods of using such proteins are described.

6 Claims, 22 Drawing Sheets

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Zuraw et al., "Clinical practice. Hereditary angioedema" N Engl J Med 359, 1027-1036, (2008).

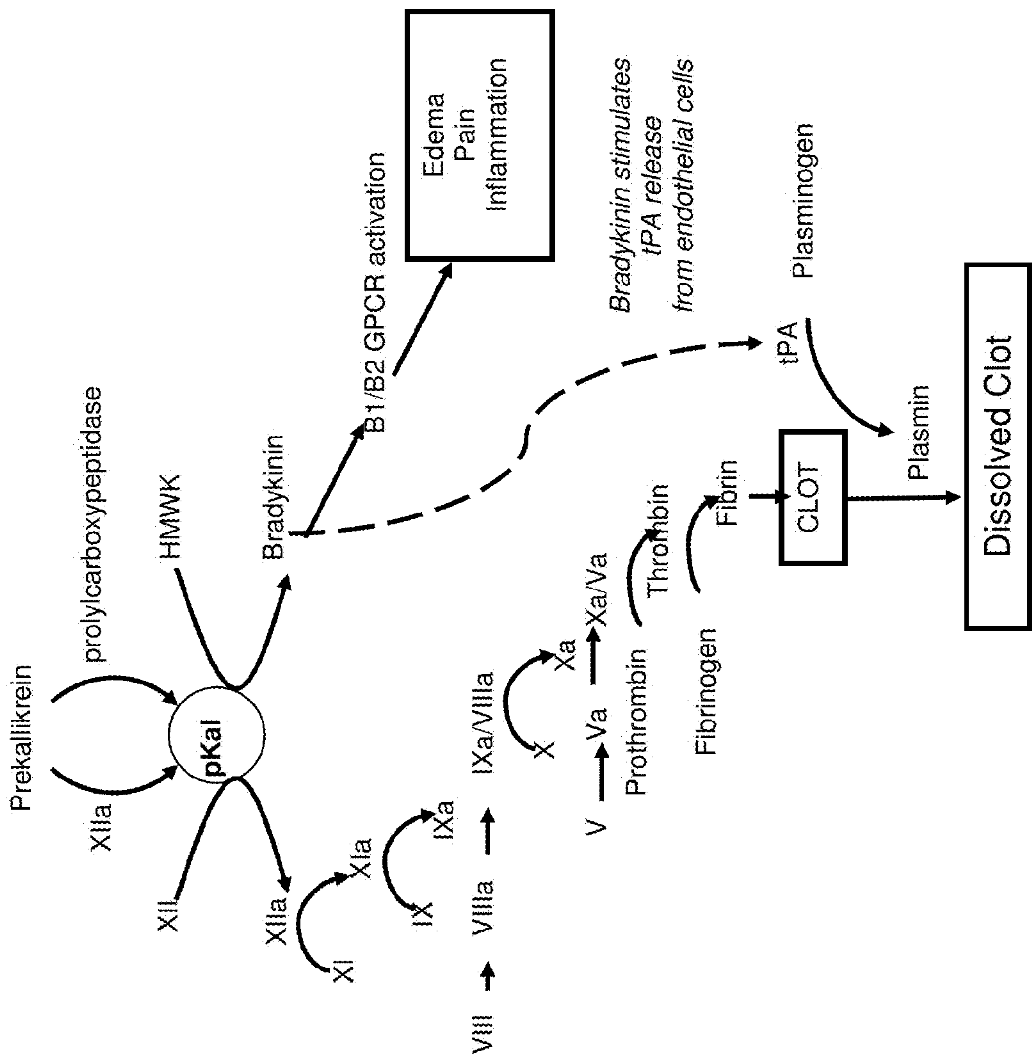
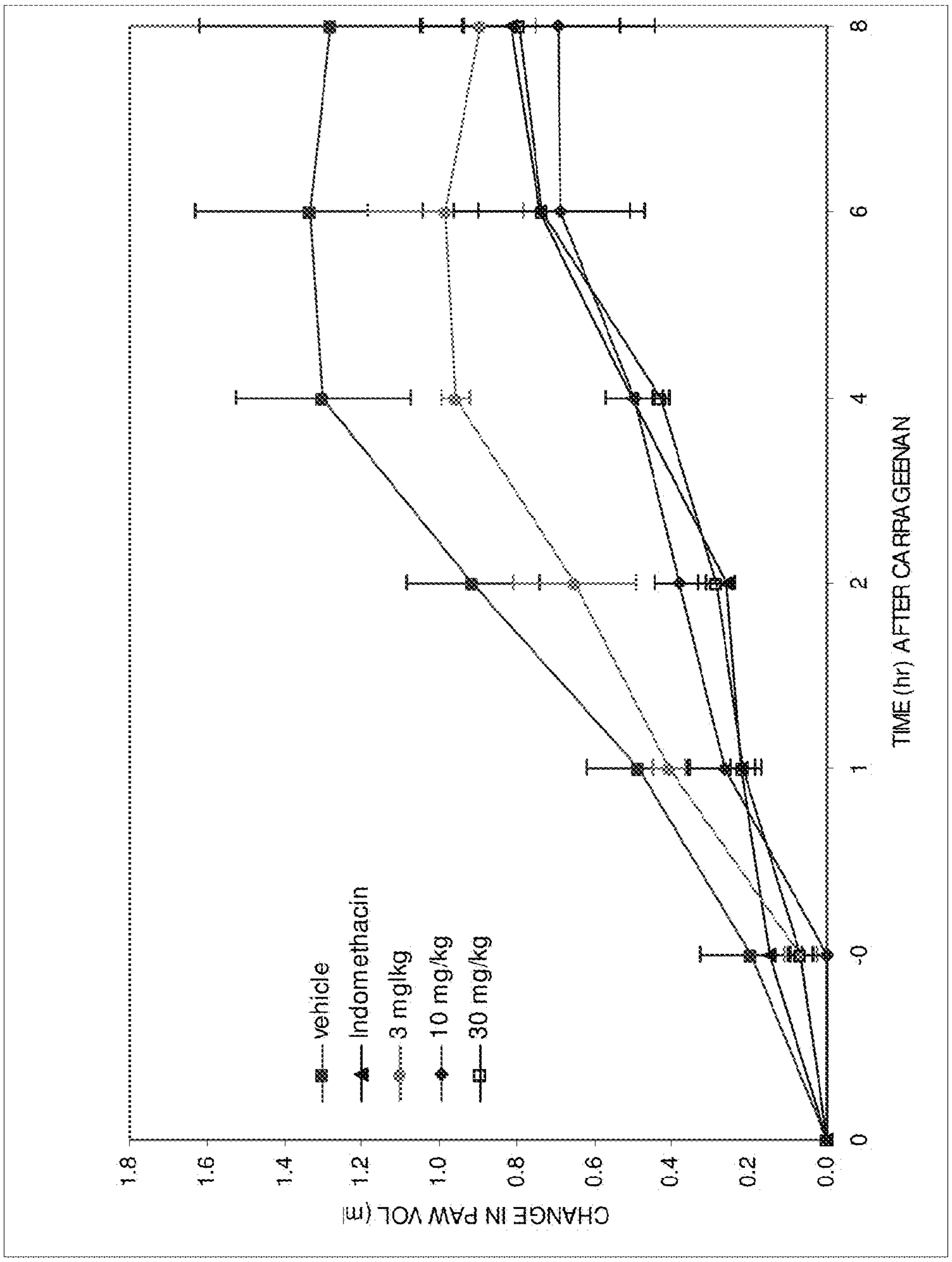

Prekallikrein
XIIa
prolylcarboxypeptidase
pKal
HMWK
Bradykinin
B1/B2 GPCR activation
Edema
Pain
Inflammation
Bradykinin stimulates
tPA release
from endothelial cells
XII
XIIa
XI
XIa
IX
IXa
VIII
VIIIa
IXa/VIIIa
X
Xa
V
Va
Xa/Va
Prothrombin
Thrombin
Fibrinogen
Fibrin
CLOT
tPA
Plasminogen
Plasmin
Dissolved Clot

FIGURE 1



vehicle
Indomethacin
3 mglkg
10 mg/kg
30 mg/kg
CHANGE IN PAW VOL (ml
TIME (hr) AFTER CARRAGEENAN
0.0
0.2
0.4
0.6
0.8
1.0
1.2
1.4
1.6
1.8
0
-0
1
2
4
6
8

FIGURE 2

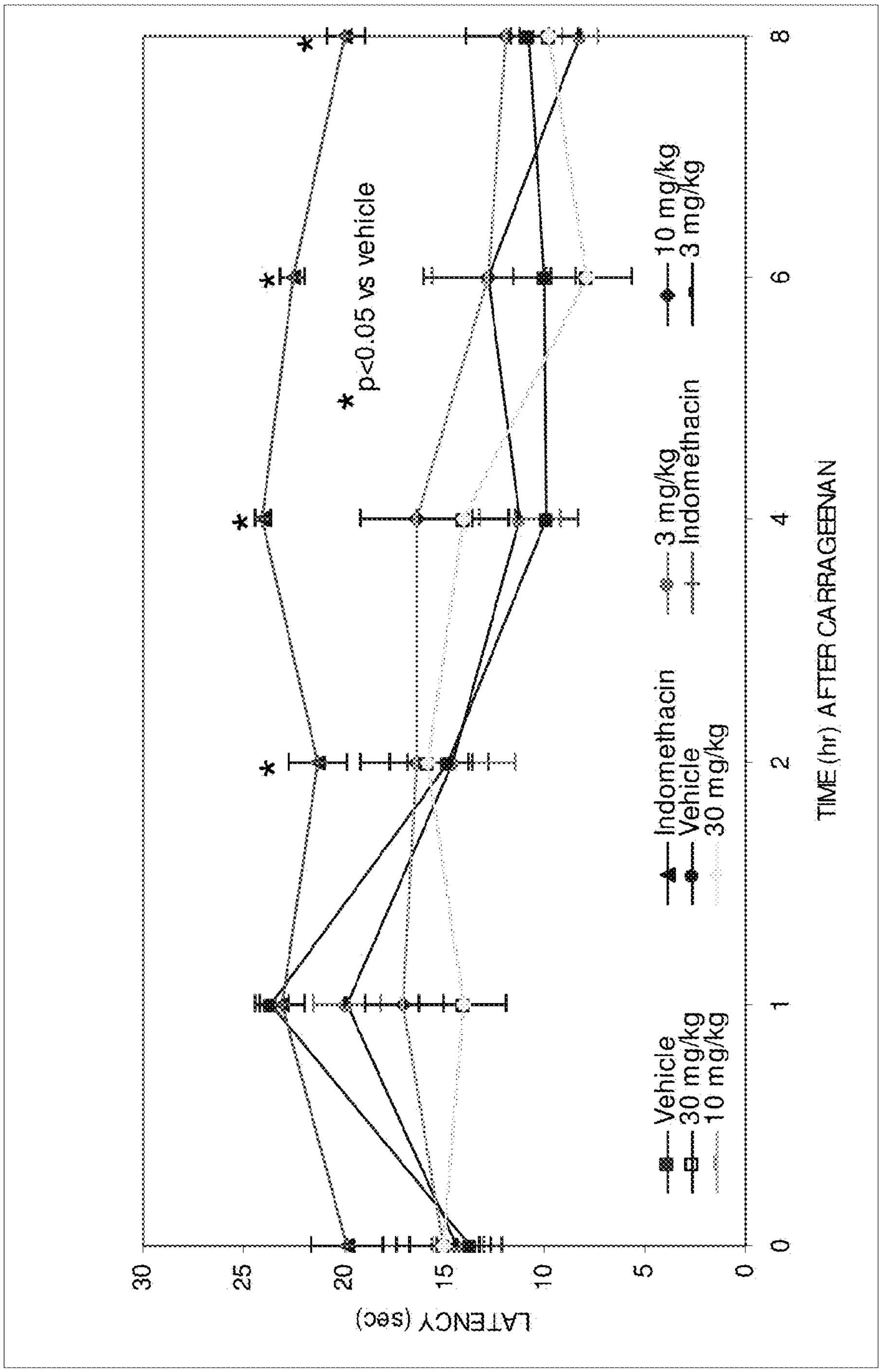

LATENCY (sec)
30
25
20
15
10
5
0
TIME (hr) AFTER CARRAGEENAN
0
1
2
4
6
8
* p<0.05 vs vehicle
Vehicle
30 mg/kg
10 mg/kg
Indomethacin
Vehicle
30 mg/kg
3 mg/kg
Indomethacin
10 mg/kg
3 mg/kg

FIGURE 3

```
X63-G06      CAAGACATCCAGATGACCCAGTCTCCAGGCACCCTGTCTTTGTCTCCAGGGGAAAGAGCC
X81-B01      ----GAGATCGTGCTGACCCAGTCCCCTGGCACCCTGTCTCTGTCTCCCGGCGAGAGAGCC
** ***  * ********** ** ************ ******* ** ** ******

X63-G06      ACCCTCTCCTGCAGGACCAGTCAATTTGTTAACAGCAACTACTTAGCCTGGTACCAACAG
X81-B01      ACCCTGTCCTGCCGGACCTCCCAGTTCGTGAACTCCAACTACCTGGCTTGGTATCAGCAG
***** ****** *****   ** ** ** ***  ******* * ** ***** ** ***

X63-G06      ACACCTGGCCAGGCTCCCAGGCTCCTCATCTATGGTGCATCCAGCAGGGCCACTGGCATC
X81-B01      AAGCCAGGCCAGGCCCCTAGACTGCTGATCTACGGCGCCTCTTCCAGAGCCACCGGCATC
*  ** ******** ** ** ** ** ***** ** ** **   *** ***** ******

X63-G06      CCAGACAGGTTCAGTGGCACTGGGTATGGGACAGACTTCACTCTCACCATCAGCAGACTG
X81-B01      CCTGACCGGTTCTCCGGCTCTGGCTCCGGCACCGACTTCACCCTGACCATCTCCCGGCTG
** *** *****   *** **** *  ** ** ******** ** ******  * * ***

X63-G06      GAGCCTGAAGATTATGGAACTTACTACTGTCAGCAGAGTTCCAGAACCCCGTGGACGTTC
X81-B01      GAACCTGAGGACTTCGCCGTGTACTACTGCCAGCAGTCCTCCCGGACCCCTTGGACCTTT
** ***** ** *  *     ******** ******   *** * ***** ***** **

X63-G06      GGCCAAGGGACCAGAGTGGAAATCAAA
X81-B01      GGCCAGGGCACCAAGGTGGAGATCAAG
***** ** ****  ***** *****
```

FIGURE 4

```
X63-G06    QDIQMTQSPGTLSLSPGERATLSCRTSQFVNSNYLAWYQQTPGQAPRLLIYGASSRATGI
X81-B01    -EIVLTQSPGTLSLSPGERATLSCRTSQFVNSNYLAWYQQKPGQAPRLLIYGASSRATGI
             *  *********************************** *******************

X63-G06    PDRFSGTGYGTDFTLTISRLEPEDYGTYYCQQSSRTPWTFGQGTRVEIK
X81-B01    PDRFSGSGSGTDFTLTISRLEPEDFAVYYCQQSSRTPWTFGQGTKVEIK
           ****** * ***************   ***************** ****
```

FIGURE 5

```
X81-B01    GAGGTGCAATTGCTGGAATCCGGCGGAGGTCTGGTGCAGCCTGGCGGCTC
X63-G06    GAAGTTCAATTGTTAGAGTCTGGTGGCGGTCTTGTTCAGCCTGGTGGTTC
           ** ** ****** * ** ** ** ** ***** ** ******** ** **

X81-B01    CCTGAGACTGTCTTGCGCCGCCTCCGGCTTCACCTTCTCCCACTACCTGA
X63-G06    TTTACGTCTTTCTTGCGCTGCTTCCGGATTCACTTTCTCTCATTACCTTA
             *  * ** ******** ** ***** ***** ***** ** ***** *

X81-B01    TGACCTGGGTGCGCCAGGCTCCTGGCAAGGGCCTCGAATGGGTGTCCTAC
X63-G06    TGACTTGGGTTCGCCAAGCTCCTGGTAAAGGTTTGGAGTGGGTTTCTTAT
           **** ***** ***** ******** ** **  * ** ***** ** **

X81-B01    ATCTCCCCCTCTGGCGGCCACACCATCTACGCCGACTCCGTGAAGGGCCG
X63-G06    ATCTCTCCTTCTGGTGGCCATACTATTTATGCTGACTCCGTTAAAGGTCG
           ***** ** ***** ***** ** ** ** ** ******** ** ** **

X81-B01    GTTCACCATCTCCCGGGACAACTCCAAGAACACCCTGTATCTGCAGATGA
X63-G06    CTTCACTATCTCTAGAGACAACTCTAAGAATACTCTCTACTTGCAGATGA
            ***** *****  * ******** ***** ** ** **  *********

X81-B01    ACTCCCTGAGGGCCGAGGACACCGCCGTGTACTACTGCGCCAGGGTGGCC
X63-G06    ACAGCTTAAGGGCTGAGGACACGGCCGTGTATTACTGTGCGAGAGTGGCC
           **  * * ***** ******** ******** ***** ** ** ******

X81-B01    AGAGGAATCGCCGCCAGGTCCCGGACCTCCTACTTCGACTACTGGGGCCA
X63-G06    CGGGGGATAGCAGCTCGATCGCGAACCAGCTACTTTGACTACTGGGGCCA
            * ** ** ** **  * ** ** ***  ****** **************

X81-B01    GGGCACCCTGGTGACCGTGTCCTCC
X63-G06    GGGAACCCTGGTCACCGTCTCAAGC
           *** ******** ***** **   *
```

FIGURE 6

```
X81-B01        EVQLLESGGGLVQPGGSLRLSCAASGFTFSHYLMTWVRQAPGKGLEWVSY
X63-G06        EVQLLESGGGLVQPGGSLRLSCAASGFTFSHYLMTWVRQAPGKGLEWVSY
               **************************************************

X81-B01        ISPSGGHTIYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARVA
X63-G06        ISPSGGHTIYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARVA
               **************************************************

X81-B01        RGIAARSRTSYFDYWGQGTLVTVSS
X63-G06        RGIAARSRTSYFDYWGQGTLVTVSS
               *************************
```

FIGURE 7

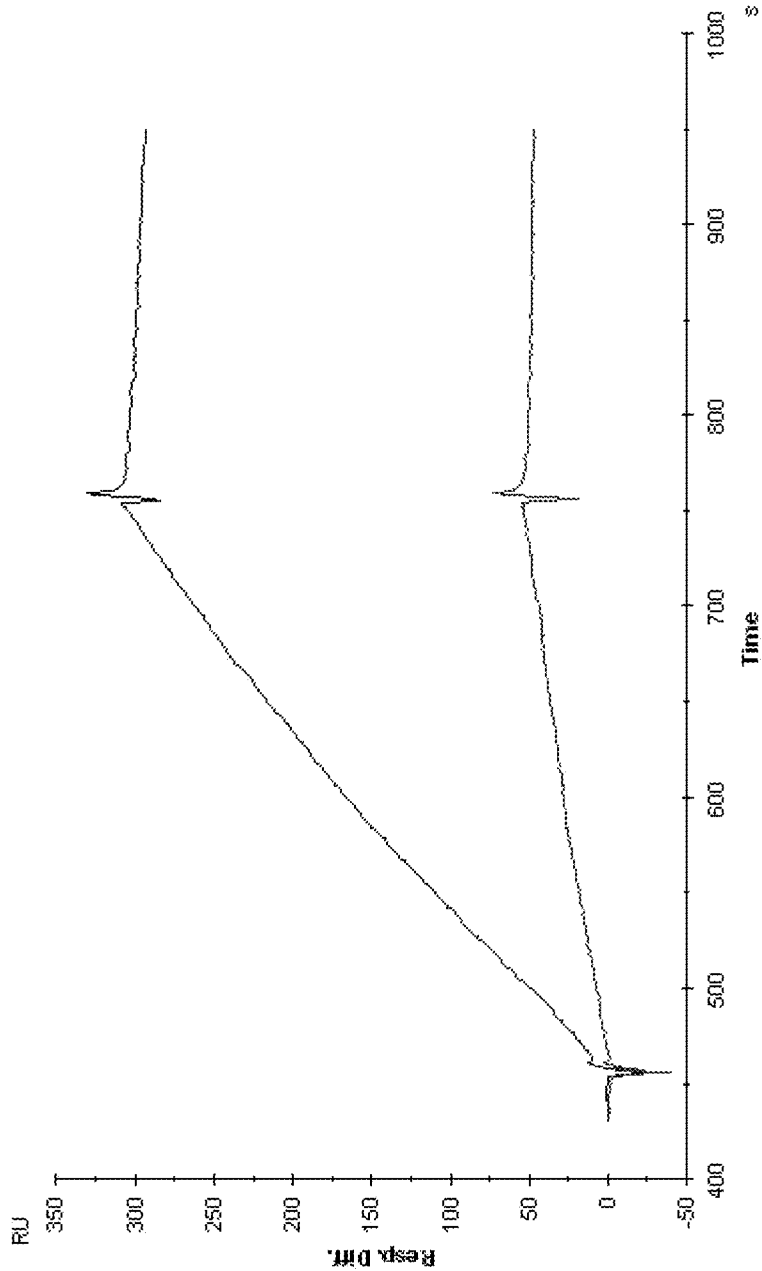
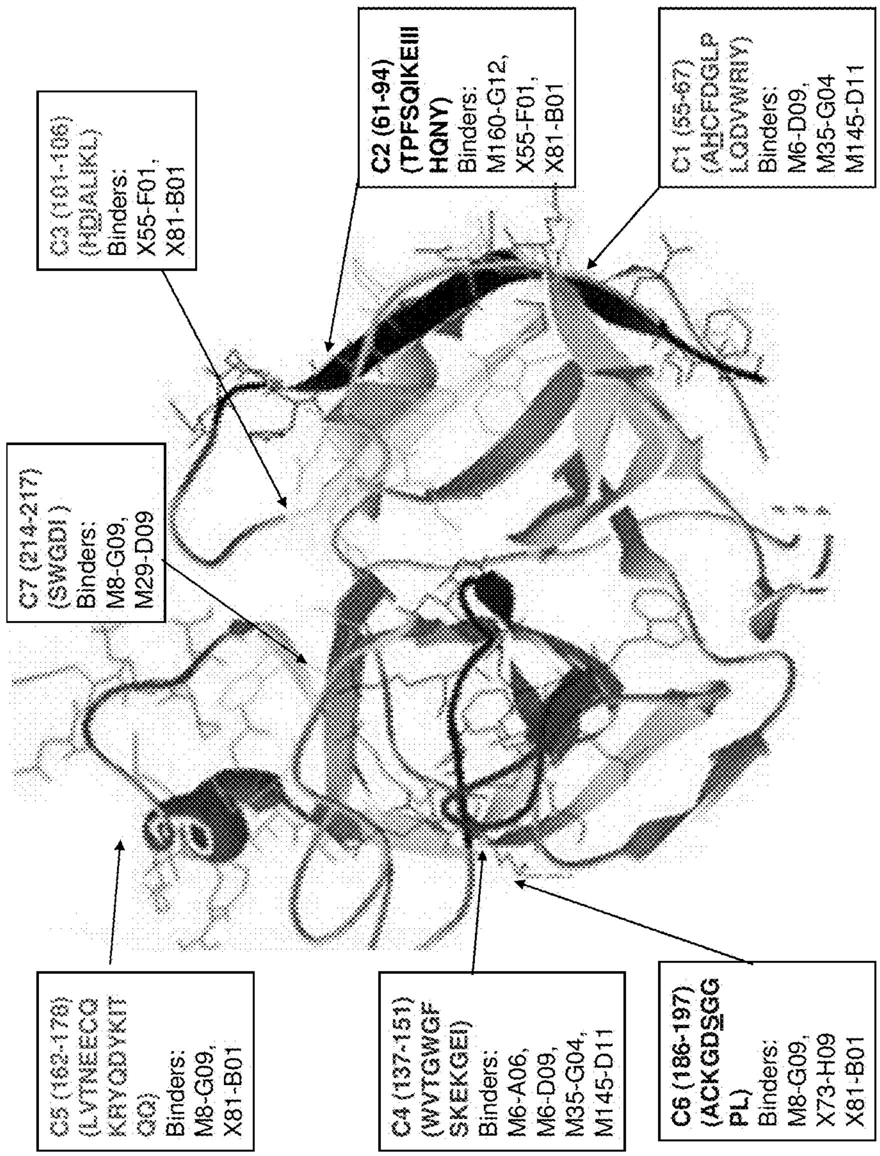

RU
Resp. Diff.
350
300
250
200
150
100
50
0
-50
400
500
600
700
800
900
1000
s
Time

FIGURE 8A


RU
Resp. Diff.
250
200
150
100
50
0
-50
400
500
600
700
800
900
1000
s
Time

FIGURE 8B



C3 (101-106) (HDIALIKL) Binders: X55-F01, X81-B01
C7 (214-217) (SWGDI) Binders: M8-G09, M29-D09
C2 (61-94) (TPFSQIKEIII HQNY) Binders: M160-G12, X55-F01, X81-B01
C1 (55-67) (AHCFDGLP LQDVWRIY) Binders: M6-D09, M35-G04 M145-D11
C5 (162-178) (LVTNEECQ KRYQDYKIT QQ) Binders: M8-G09, X81-B01
C4 (137-151) (WVTGWGF SKEKGEI) Binders: M6-A06, M6-D09, M35-G04, M145-D11
C6 (186-197) (ACKGDSGG PL) Binders: M8-G09, X73-H09, X81-B01

FIGURE 9

```
Human    --------MILFKQATYFISLFATVSCGCLTQLYENAFFRGGDVASMYTPNAQYCQMRCT 52
Cow      --------MIALRQAAYFICLFATVSCGCLTQLYHNIFFRGGDVSAMYTPDAQYCQLMCT 52
Mouse    --------MILFNRVGYFVSLFATVSCGCMTQLYKNTFFRGGDLAAIYTPDAQYCQKMCT 52
Rat      --------MILFKQVGYFVSLFATVSCGCLSQLYANTFFRGGDLAAIYTPDAQHCQKMCT 52
Pig      MEVIVLFRIISFRQAVYFMCLFAAVSCGCLPQLHKNTFFRGGDVSAMYTPSARHCQMMCT 60
Frog     --------MACYYSSLLFLLLFTLVSGGCISELYQDIYWQGGDLRSVFAPDVEYCQLVCT 52
MACACA   --------MILFKQATYFISLFATVSCGCLTQLYENAFFRGGDVASMYTPNAQHCQMMCT 52
Dog      ---------MLIKLP------LKWVSQGCLTQLYKNTFFKGGDLTAMYTPNAHHCQMMCT 45
                             :  ** **:.:*: : :::***: ::::*...:**  **

Human    FHPRCLLFSFLPASSINDMEKRFGCFLKDSVTGTLPKVHRTGAVSGHSLKQCGHQISACH 112
Cow      FHPRCLLFSFLPENSTSDADKRFGCFLKDSVTGTLPRVSRTGAISGHSLKRCGHQISACH 112
Mouse    FHPRCLLFSFLAVTPPKETNKRFGCFMKESITGTLPRIHRTGAISGHSLKQCGHQISACH 112
Rat      FHPRCLLFSFLAVSPTKETDKRFGCFMKESITGTLPRIHRTGAISGHSLKQCGHQLSACH 112
Pig      FHPRCLLFSFLPADSTSVTDKRFGCFLKDSVTGMLPRVLRENAISGHSLKQCGHQIRACH 120
Frog     FSPRCLMFSYLPASWPK-ENERFACYLKESATNMLPKVTLTGVISGHSLKNCKSKINVCR 111
MACACA   FHPRCLLFSFLPASSINDMEKRFGCFLKDSVTGTLPKVRRAGAISGHSLKQCGHQISACH 112
Dog      FHPRCLLFSFLPESSTNDVNKRFGCFLKDSVTETLPRMSWTSAISGHSLKQCGHQISACH 105
         * ****:**:*.      .   ::**.*::*:* *  **::    ..:******.*  :: .*:

Human    RDIYKGVDMRGVNFNVSKVSSVEECQKRCTNNIRCQFFSYATQTFHKAEYRNNCLLKYSP 172
Cow      RSIYKGIDMRGVNFNASKVRSAKECQERCTNNIHCQFFTYATKTFFSAEYRNTCLLKRSP 172
Mouse    RDIYKGLDMRGSNFNISKTDNIEECQKLCTNNFHCQFFTYATSAFYRPEYRKKCLLKHSA 172
Rat      QDIYEGLDMRGSNFNISKTDSIEECQKLCTNNIHCQFFTYATKAFHRPEYRKSCLLKRSS 172
Pig      RDIYKGIDMRGVNFNVSKVKTVEECQERCTNSIHCLFFTYATQAFNNAEYRNNCLLKHSP 180
Frog     DKNFPGIDMIGTNYNVTSTANVQQCKEGCTNDIYCQYFTYVTQEFHSAQLRNRCYFKYSG 171
MACACA   RDIYKGIDMRGVNFNVSKVSSVEECQKRCTNNIRCQFFSYATQTFHNAEYRNTCLLKHSP 172
Dog      RDIHKGIDMRGVNFNVSKVKSVEECQKKCTNSIHCQFFTYATETFYNVEYRNSCLLKNGP 165
           . .  *:** * *:* :.. . ::*:: ***.: * :*:*.*. *    : *: * :* .

Human    GGTPTAIKVLSNVESGFSLKPCALSEIGCHMNIFQHLAFSDVDVARVLTPDAFVCRTICT 232
Cow      QGTPTRIKVLSDVESGFSLKACGNSKIGCRVDIFQHSAFSDVDVAGIIAPDAFVCRTICT 232
Mouse    SGTPTSIKSADNLVSGFSLKSCALSEIGCPMDIFQHSAFADLNVSQVITPDAFVCRTICT 232
Rat      SGTPTSIKPVDNLVSGFSLKSCALSEIGCPMDIFQHFAFADLNVSQVVTPDAFVCRTVCT 232
Pig      GGTPTSIKVLANVESGFSLKPCADSEIGCHMDIFQHLAFSDVDVARVIAPDAFVCRTICT 240
Frog     KGMPTRIRLLDNVISGFSLKACGKSSLGCQNDLFQNMELPGETLTRVFAPDVLTCQKICT 231
MACACA   GGTPTTIKVLNNVESGFSLKPCALSEIGCHMNIFQHLAFSDVDVARVLAPDAFVCRTICT 232
Dog      GGTPSSIKVLADVVSGFSLKSCALSEIGCHMNIFQHLAFSDVDVARVVTPDAFVCQTICT 225
          * *: *:    :: ******.*. *.:**  ::**:   :..  :: :.:**.:.*:.:**
```

FIGURE 10A

```
Human    YHPNCLFFTFYTNVWKIESQRNVCLLKTSESGTPSSSTPQE----NTISGYSLLTCKRTLP 289
Cow      YHPSCLFFTFYTNAWKTDSQRNVCFLKTSQSGSPSSPTPQE---NAISGYSLLTCKQTLP 289
Mouse    FHPNCLFFTFYTNEWETESQRNVCFLKTSKSGRPSPPIPQE---NAISGYSLLTCRKTRP 289
Rat      FHPNCLFFTFYTNEWETESQRNVCFLKTSKSGRPSPPIIQE---NAVSGYSLFTCRKARP 289
Pig      YHPNCLFFTFYTNAWKIESQRNVCFLKTSHSGTPSFPTPQE---NAISGYSLLTCKQTLP 397
Frog     FYPNCLFFTFFKKDSKDPLQRNVCYVRTSTKGIPDEVINKE----HTISGFSLLSCKFSPS 288
MACACA   YHPSCLFFTFYTNAWKIESQRRVCMLAGSQDGAAHSSLGDARLRLKQKNKNKQTKKNTLP 292
Dog      YHPNCLFFTFYTKAWHLEPQRNVCFLKTSKSGTPSSPTSQK---NAMSGYSLLTCKKALP 282
         ::*.******:.:   .    **.**  :   * .* .      .      .. .  : : : .

Human    --EPCHSKIYPGVDFGGEELNVTFVKGVNVCQETCTKMIRCQFFTYSLLPEDCKEEKCKC 347
Cow      GTEPCHSKIYPQVAFEGEELHVTFVKGVDGCQETCTKMIRCQFFTYSLFPEDCRGEKCKC 349
Mouse    ---EPCHSKIYSGVDFEGEELNVTFVQGADVCQETCTKTIRCQFFIYSLLPQDCKEEGCKC 347
Rat      ---EPCHFKIYSGVAFEGEELNATFVQGADACQETCTKTIRCQFFTYSLLPQDCKAEGCKC 347
Pig      --EPCHSKIYSEVDFEGEELNVTFVQGANLCQETCTKTIRCQFFTYSLHPEDCRGEKCKC 355
Frog     ---VCPLTMLSDSEFLGDELLVEEVSGEKECQQACTNNIRCQFFTYGPVKSGCLEKKCKC 345
MACACA   --EPCHSKIYPGVDFGGEELNVTFVKGVNVCQETCTKMIRCQFFTYSLLPEDCKEEKCKC 350
Dog      --EPCHSKIYSGVDFEGEELNVTFAEGVNACQETCTKMIRCQFFTYSLRPEDCRGEKCKC 340
              *   .:  .    * *:**  .   ..* . **::**: ****** *.    ..*  : ***

                                                  |Start Catalytic Domain
Human    FLRLSMDGSPTRIAYGTQGSSGYSLRLCNTGDNSVCT--TKTSTRIVGGTNSSWGEWPWQ 405
Cow      SLRLSLDGSPTNITYGTQASSGYSLRLCKRGDSRVCT--TKR-TRIVGGTNASWGEWPWQ 406
Mouse    SLRLSTDGSPTRITYGMQGSSGYSLRLCKLVDSPDCT--TKINARIVGGTNASLGEWPWQ 405
Rat      SLRLSTDGSPTRITYEAQGSSGYSLRLCKVVESSDCT--TKINARIVGGTNSSLGEWPWQ 405
Pig      SLRLSSDGSPTKITHGMRASSGYSLRLCRSGDHSACA---TKANTRIVGGTDSFLGEWPWQ 413
Frog     HMKISSNGLPTGIRHGNGGISGFSLRLCKMKSVKGCGEPSEHANRIVGGTDSVLGEWPWQ 405
MACACA   FLRLSSDGSPTRITYGTQGSSGYSLRLCNTGDSSVCT--TKTSSRIVGGTNSSWGEWPWQ 408
Dog      SLRLSLDGSPTGMTYGTRVSSGYSLRLCKSGDSSVCT--TKTSTRIVGGTNSSWGEWPWQ 398
          :::* :* ** : :     **:*****.  .   *    ::    ******::  ******

          @@@@@@@@@@@@@@            #@@@@@@@@@@@@@
                                   +++++++++++++++(C1)      ++
Human    VSLQVKSTA--QSHLCGGSLIGHQWVLTAAHCFDGLPLQDVWRIYSGILNLSDITKDTPF 463
Cow      VSLQVKQRA--QSHLCGGSIIGRQWVLTAAHCFDGLLLSNIWRIYGGILNLSEITTETSF 464
Mouse    VSLQVKLVS--QTHLCGGSIIGRQWVLTAAHCFDGIPYPDVWRIYGGILSLSEITKETPS 463
Rat      VSLQVKLVS---QNHMCGGSIIGRQWILTAAHCFDGIPYPDVWRIYGGILNLSEITNKTPF 463
Pig      VSLQAKLRA---QNHLCGGSIIGHQWVLTAAHCFDGLSLPDIWRIYGGILNISEITKETPF 471
Frog     VSMHLRLGASYKKHACGGSIISNQWIVTAAHCVALYPQPQMWIIYSGFVRILNITKSTPF 465
MACACA   VSLQVKLMA--QRHLCGGSLIGHQWVLTAAHCFDGLPLPDVWRIYSGILNLSDITKETPF 466
Dog      VSLQVKLRA--QSHLCGGSIIGRQWVLTAAHCFDELSLPDVWRIYSGILNLSEITKETPF 456
         **:: :  :   : * ****:*..**::*****.      ::* **.*:: : :**..*.
```

FIGURE 10B

```
                    @@@@@@@@@@@@@
              ++++++++++++(C2)   ++++++++(C3)                        +++++
Human         SQIKEIIIHQNYKVSEG-NHDIALIKLQAPLNYTEFQKPICLPSKGDTSTIYTNCWVTGW 522
Cow           SQIKEIIVHPNYKISEG-SHDIALIKLEAPLNFTDLQKAICLPSKDDTKPVYTDCWITGW 523
Mouse         SRIKELIIHQEYKVSEG-NYDIALIKLQTPLNYTEFQKPICLPSKADTNTIYTNCWVTGW 522
Rat           SSIKELIIHQKYKMSEG-SYDIALIKLQTPLNYTEFQKPICLPSKADTNTIYTNCWVTGW 522
Pig           SQVKEIIIHQNYKILES-GHDIALLKLETPLNYTDFQKPICLPSRDDTNVVYTNCWVTGW 530
Frog          SELEKIIIHPHYTGAGN-GSDIALLKLKTPIVFNDHQKAICLPPSEATLVLPNSCWITGW 524
MACACA        SQIKEIIIHQNYRISEG-NHDIALIKLQAPLNYTEFQKPICLPSKGDTNTIYTNCWVTGW 525
Dog           SQIKEIIIHQNYKITDGGSYDIALIKLEAPLNYTEFQKPICLPSKDDTNTTYTNCWVTGW 516
              *  :::*:* .*     . . ****:**::*: :.: **.****.    *    ..**:***

              @@@@@@@@@@@@            @@@@@@@@@@@@@
              +++++++++(C4)       ++++++++++++++++++(C5)      ++++++++++(C6)
Human         GFSKEKGEIQNILQKVNIPLVTNEECQKRYQDYKITQQMVCAGYKEGGKDACKGDSGGPL 582
Cow           GFTEEKGKIQNTLQKANIPLISNEECQKSYRDYKITKQMICAGYKEGGKDACKGDSGGPL 583
Mouse         GYTKEQGETQNILQKATIPLVPNEECQKKYRDYVINKQMICAGYKEGGTDACKGDSGGPL 582
Rat           GYTKERGETQNILQKATIPLVPNEECQKKYRDYVITKQMICAGYKEGGIDACKGDSGGPL 582
Pig           GFTEEKGEIQNILQKVNIPLVSNEECQKSYRDHKISKQMICAGYKEGGKDACKGESGGPL 590
Frog          GYTEETGSPGNVLQKAEVPPISTEECQGSYVETRIDKKVLCAGYKSGKIDACKGDSGGPL 584
MACACA        GFSKEKGEIQDILQKVNIPLVTNEECQKRYQDYKITQRMVCAGYKEGGKDACKGDSGGPL 585
Dog           GFTKERGEIQNSLQKANIPLVPNEECQKKYRDYEVNKQMICAGYKEGGKDACKGDSGGPL 576
              *:::* *.  : ***. :* :..****  * :   : :::*****.*  *****:*****

                            @@@@@@@@@@@@
                            ++++(C7)
Human         VCKHNGMWRLVGITSWGEGCARREQPGVYTKVAEYMDWILEKTQSSDGKAQMQSPA 638
Cow           VCQHEETWHLVGITSWGEGCARREQPGVYTKVAEYVDWILEKTQDSHGQPLRK--- 636
Mouse         VCKHSGRWQLVGITSWGEGCGRKDQPGVYTKVSEYMDWILEKTQSSDVRALETSSA 638
Rat           VCKHSGRWQLVGITSWGEGCARKEQPGVYTKVAEYIDWILEKIQSSKERALETSPA 638
Pig           VCKYNGIWHLVGTTSWGEGCARREQPGVYTKVIEYMDWILEKTQDDDGQSWMK--- 643
Frog          VCEVDEIWYLTGITSWGEGCARPGKPGVYTRVSTFINWILEHTKL----------- 629
MACACA        ACKHNGMWRLVGITSWGEGCARREQPGVYTKVAEYMDWILEKTQSSDGNARMQAPA 641
Dog           VCKHNGNWHLVGITSWGEGCGRREQPGVYTKVAEYVDWILEKTQVGDGHAGLG--- 629
              .*: .    * *.* *******.*   :*****:*   : :****: :
```

FIGURE 10C

NOTE: The underlined positions are the amino acids that form the catalytic triad (His434, Asp483, and Ser578, numbering based on the human sequence).

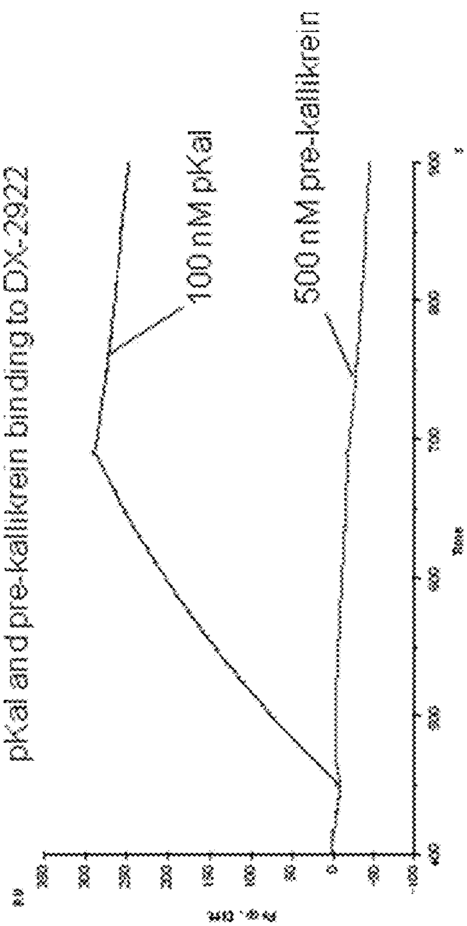

pKal and pre-kallikrein binding to DX-2922
100 nM pKal
500 nM pre-kallikrein

FIGURE 11A

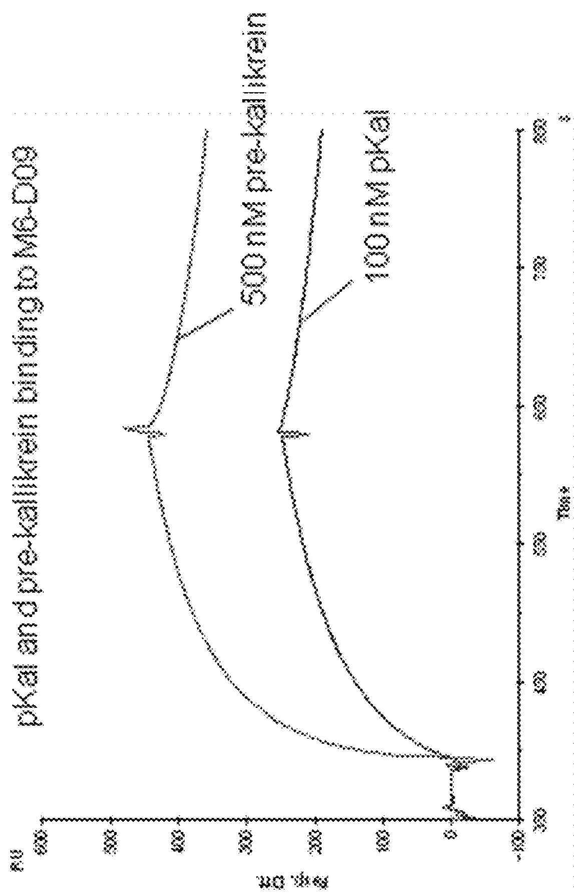

pKal and pre-kallikrein binding to M6-D09
500 nM pre-kallikrein
100 nM pKal

FIGURE 11B

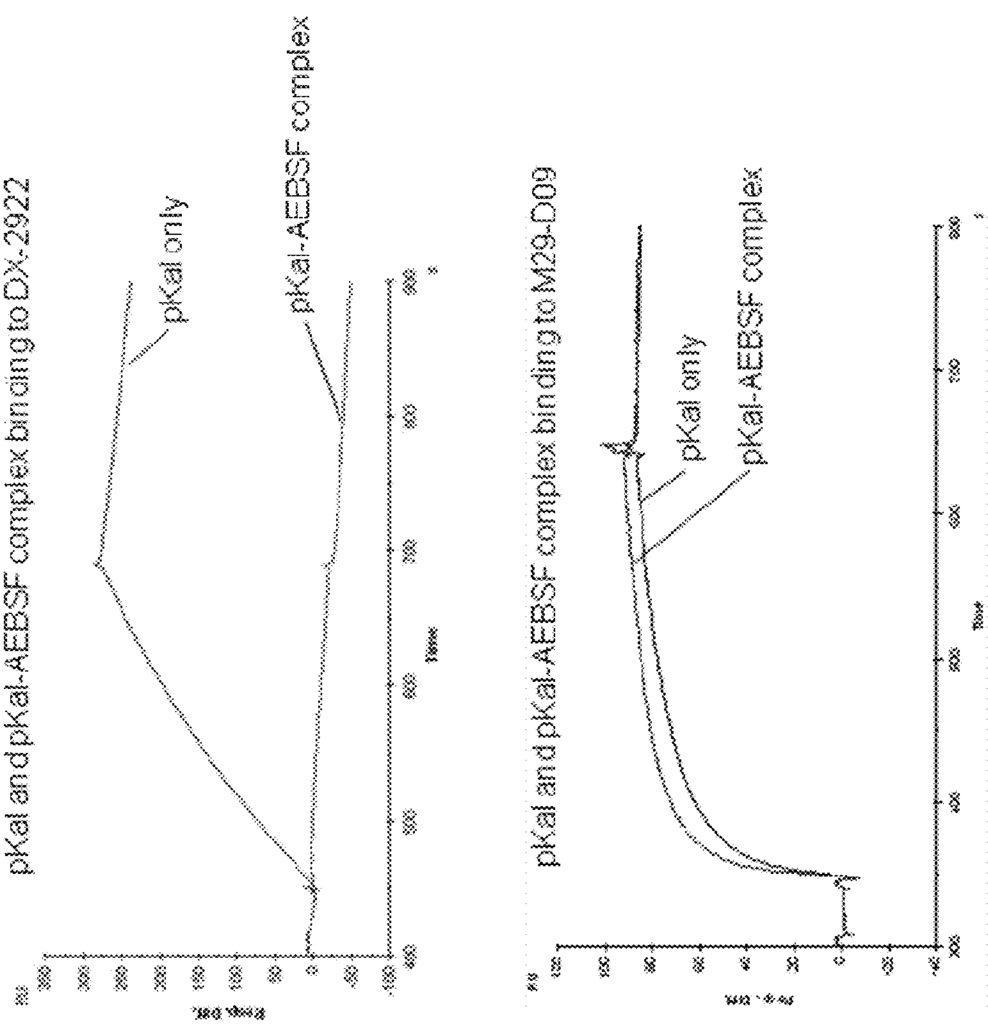

pKal and pKal-AEBSF complex binding to DX-2922
pKal only
pKal-AEBSF complex
pKal and pKal-AEBSF complex binding to M29-D09
pKal only
pKal-AEBSF complex

FIGURE 12

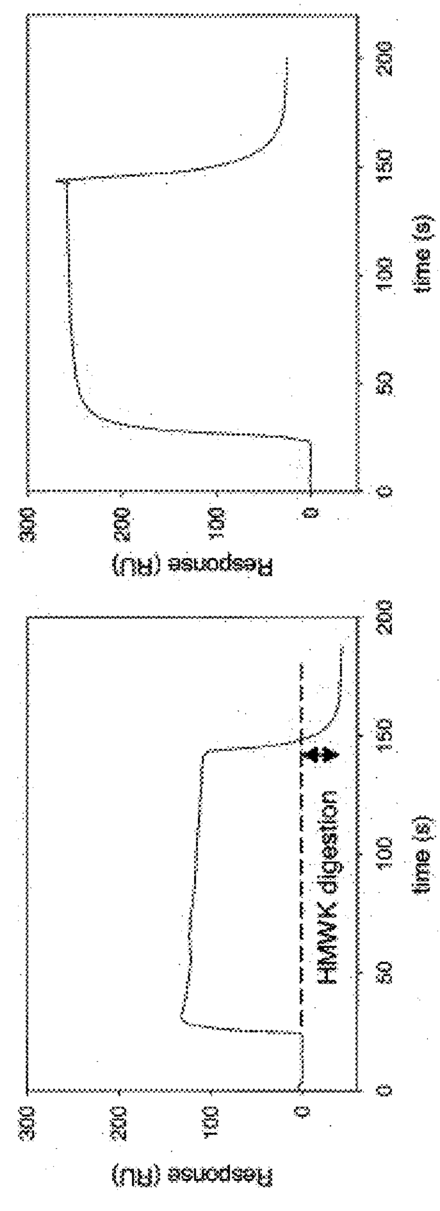


FIGURE 13

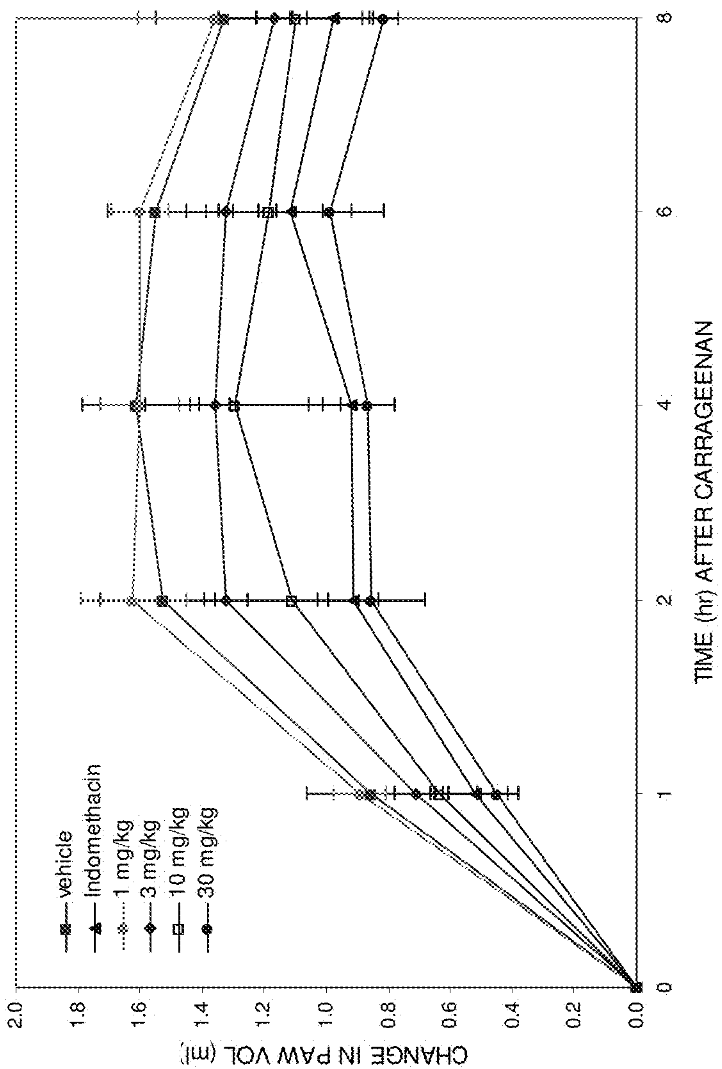

vehicle
Indomethacin
1 mg/kg
3 mg/kg
10 mg/kg
30 mg/kg
CHANGE IN PAW VOL (ml)
2.0
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
0
1
2
4
6
8
TIME (hr) AFTER CARRAGEENAN

FIGURE 14

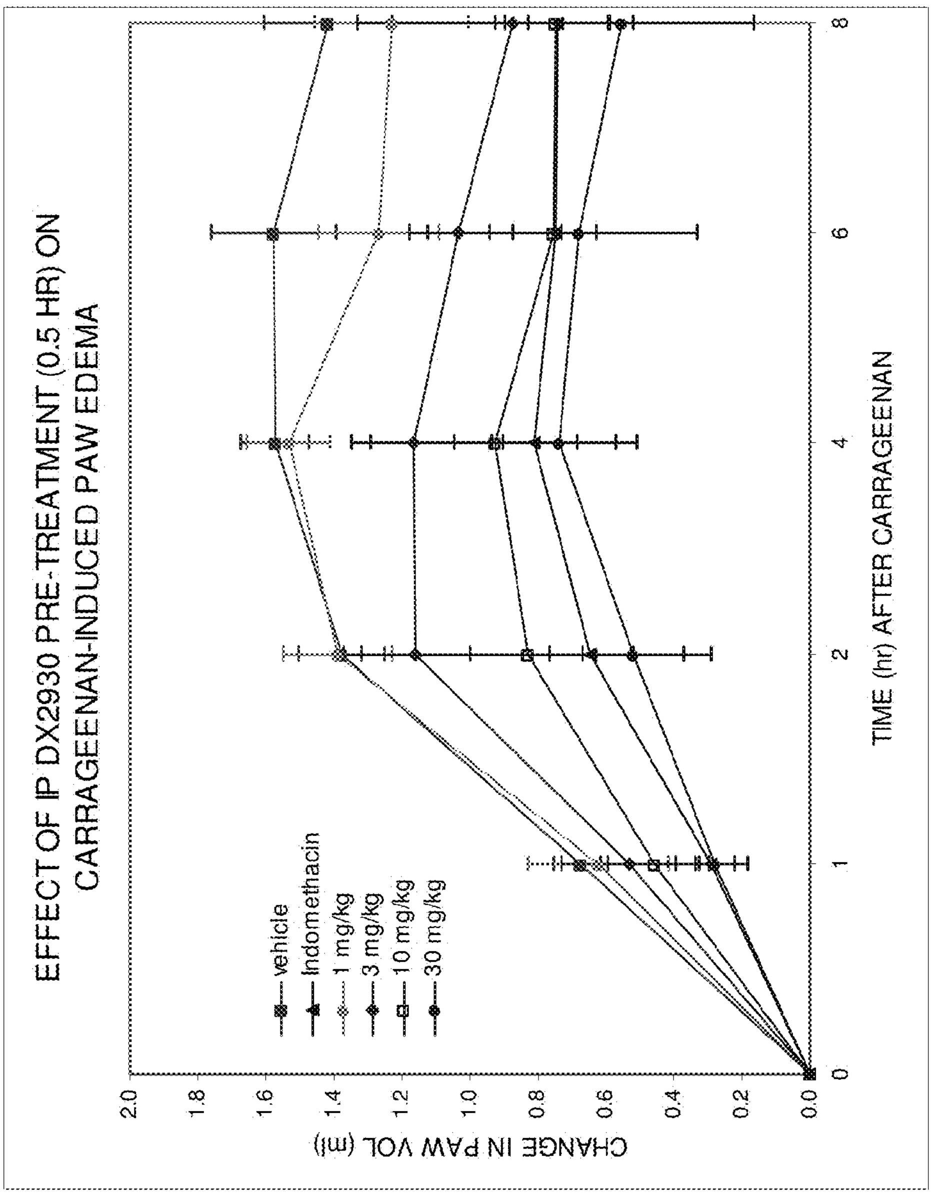

EFFECT OF IP DX2930 PRE-TREATMENT (0.5 HR) ON
CARRAGEENAN-INDUCED PAW EDEMA
vehicle
Indomethacin
1 mg/kg
3 mg/kg
10 mg/kg
30 mg/kg
CHANGE IN PAW VOL (ml)
0.0
0.2
0.4
0.6
0.8
1.0
1.2
1.4
1.6
1.8
2.0
0
1
2
4
6
8
TIME (hr) AFTER CARRAGEENAN

FIGURE 15

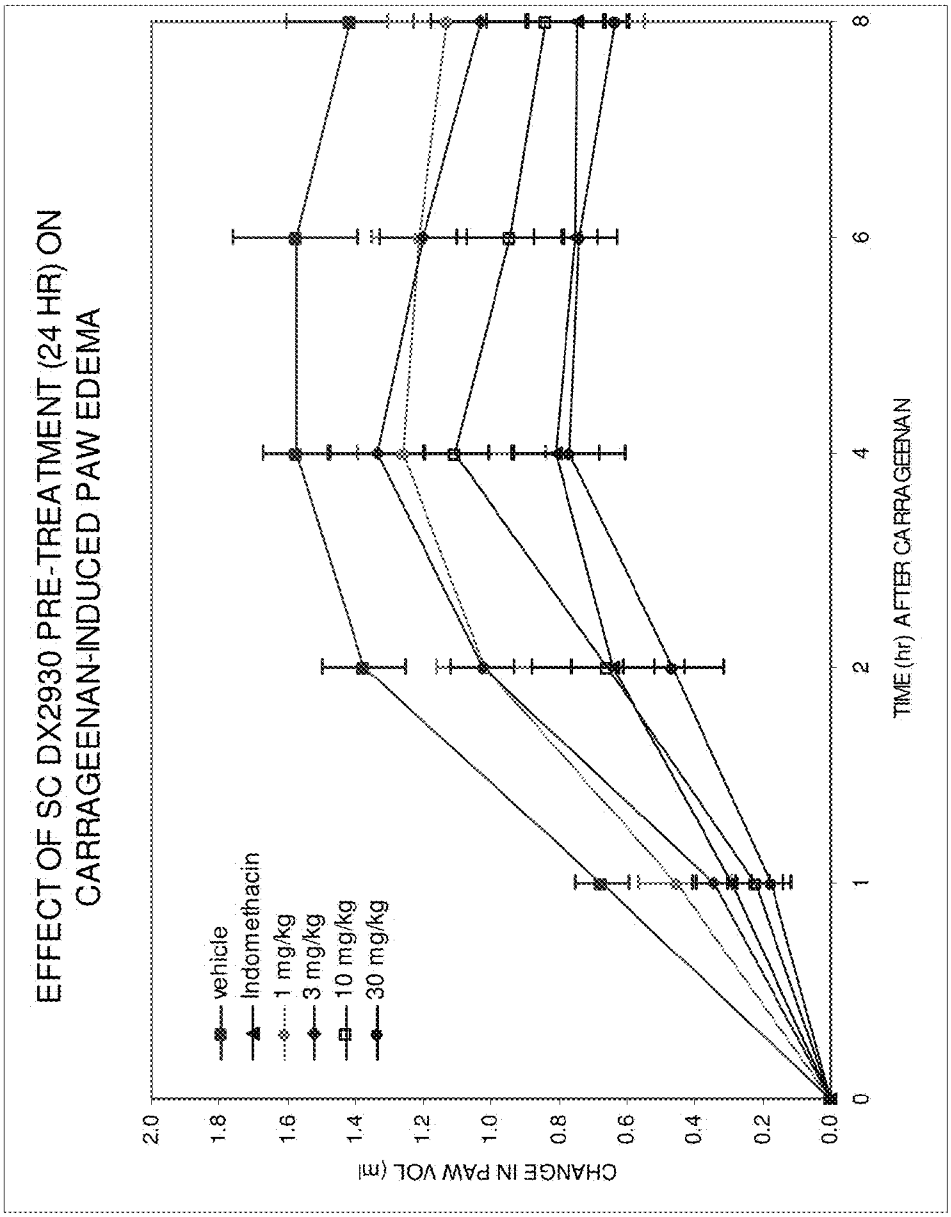
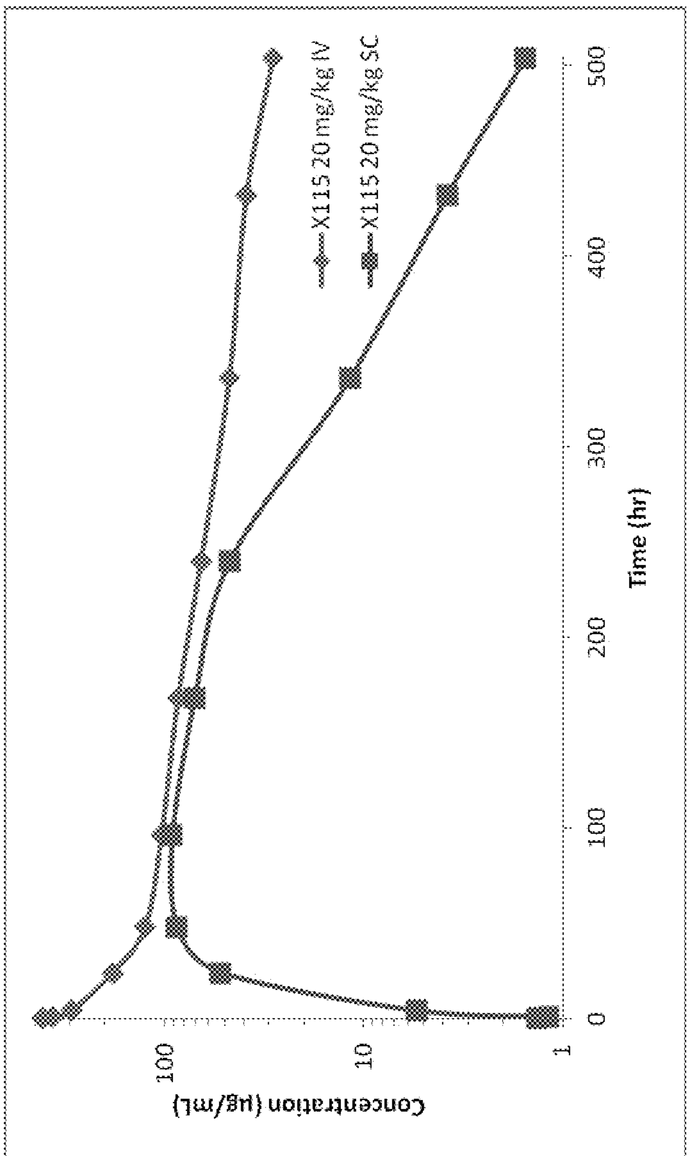

EFFECT OF SC DX2930 PRE-TREATMENT (24 HR) ON CARRAGEENAN-INDUCED PAW EDEMA
vehicle
Indomethacin
1 mg/kg
3 mg/kg
10 mg/kg
30 mg/kg
CHANGE IN PAW VOL (ml)
2.0
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
0
1
2
4
6
8
TIME (hr) AFTER CARRAGEENAN

FIGURE 16



Concentration (μg/mL)
100
10
1
0
100
200
300
400
500
Time (hr)
X115 20 mg/kg IV
X115 20 mg/kg SC

FIGURE 17

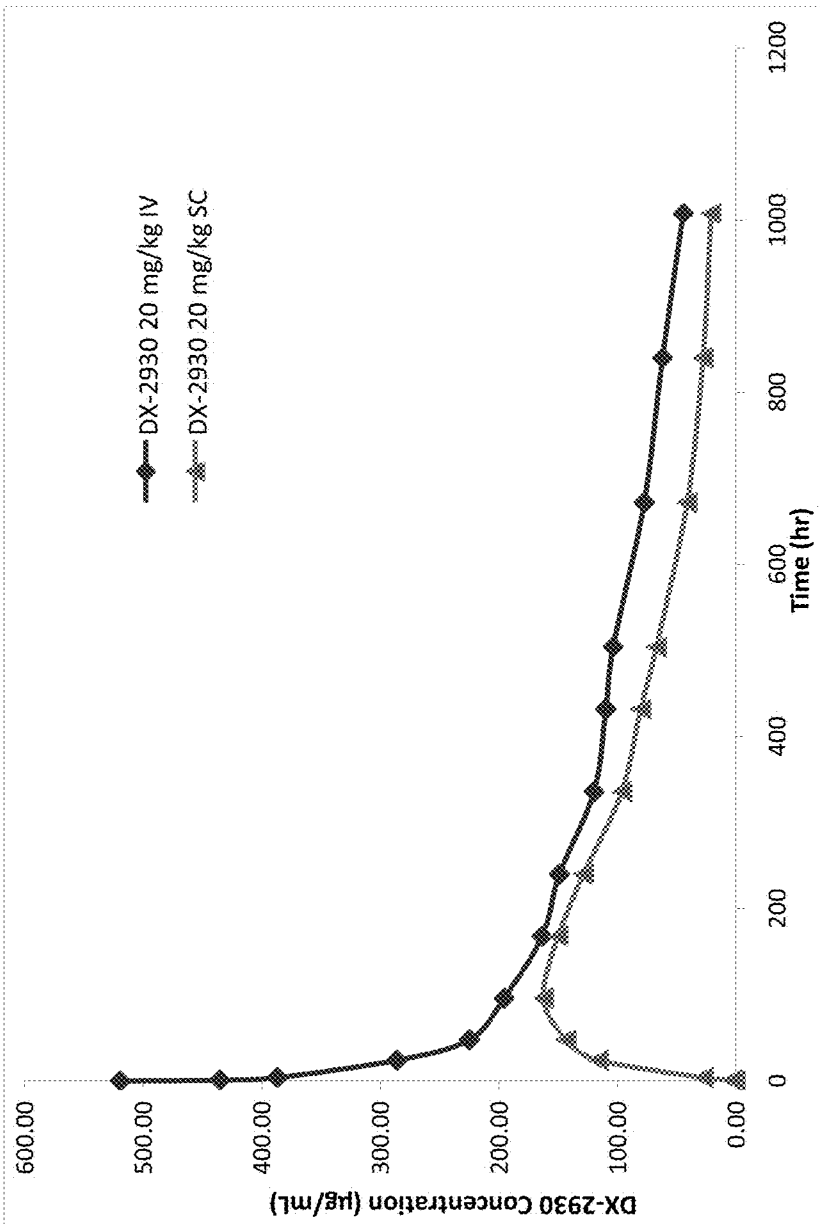

DX-2930 20 mg/kg IV
DX-2930 20 mg/kg SC
DX-2930 Concentration (µg/mL)
Time (hr)
600.00
500.00
400.00
300.00
200.00
100.00
0.00
0
200
400
600
800
1000
1200

FIGURE 18

METHOD OF TREATING HEREDITARY ANGIOEDEMA USING PLASMA KALLIKREIN BINDING ANTIBODIES

This application is a divisional of U.S. application Ser. No. 13/345,170, filed on Jan. 6, 2012, which claims priority to U.S. Application Ser. No. 61/430,442, filed on Jan. 6, 2011. The disclosure of the prior application is considered part of and is incorporated by reference in the disclosure of this application.

BACKGROUND

Plasma kallikrein is a serine protease. Prekallikrein is the precursor of plasma kallikrein.

SUMMARY

Plasma kallikrein is a serine protease component of the contact system and a potential drug target for different inflammatory, cardiovascular, infectious (sepsis) and oncology diseases (Sainz I. M. et al., Thromb Haemost 98, 77-83, 2007). The contact system is activated by either factor XIIa upon exposure to foreign or negatively charged surfaces or on endothelial cell surfaces by prolylcarboxypeptidases (FIG. **1**) (Sainz I. M. et al., Thromb Haemost 98, 77-83, 2007). Activation of the plasma kallikrein amplifies intrinsic coagulation via its feedback activation of factor XII and enhances inflammation via the production of the proinflammatory nonapeptide bradykinin. As the primary kininogenase in the circulation, plasma kallikrein is largely responsible for the generation of bradykinin in the vasculature. A genetic deficiency in the C1-inhibitor protein (C1-INH), the major natural inhibitor of plasma kallikrein, leads to hereditary angioedema (HAE). Patients with HAE suffer from acute attacks of painful edema often precipitated by unknown triggers (Zuraw B. L. et al., N Engl J Med 359, 1027-1036, 2008). Through the use of pharmacological agents or genetic studies in animal models, the plasma kallikrein-kinin system (plasma KKS) has been implicated in various diseases.

Plasma kallikrein binding proteins (e.g., antibodies, e.g., inhibitory antibodies) are useful therapeutic agents for a variety of diseases and conditions, e.g., diseases and conditions that involve plasma kallikrein activity, due to their high potency, specificity, and prolonged serum residency. High potency can translate to efficacy and a low drug dosage, and high specificity can reduce side effects due to the inhibition of related off target serine proteases. In general, small molecule serine proteases are not as specific as antibody inhibitors. Prolonged serum residency can permit infrequent dosing.

In some aspects, the disclosure features an isolated protein (e.g., antibody, e.g., human antibody) that binds to the active form of plasma kallikrein (e.g., human plasma kallikrein and/or mouse plasma kallikrein), and, e.g., does not bind pre-plasma kallikrein (e.g., human preplasma kallikrein and/or mouse preplasma kallikrein).

In some embodiments, the plasma kallikrein binding protein binds the same epitope or competes for binding with a kallikrein binding protein described herein. In some embodiments, the plasma kallikrein binding protein binds the same epitope or competes for binding with a protein (e.g., epi-Kal2) and/or a small molecule (e.g., AEBSF) described herein and does not bind pre-plasma kallikrein.

In some embodiments, the protein described herein is selected from the group consisting of M162-A04, M199-A08, M160-G12, M142-H08, X63-G06, X101-A01 (also referred to herein as DX-2922), X81-B01, X67-D03, X67-G04, X115-B07, X115-D05, X115-E09, X115-H06, X115-A03, X115-D01, X115-F02, X124-G01 (also referred to herein as DX-2930), X115-G04, M29-D09, M145-D11, M06-D09 and M35-G04.

In some embodiments, the plasma kallikrein binding protein competes with or binds the same epitope as X81-B01 and, e.g., does not bind pre-plasma kallikrein.

In some embodiments, the plasma kallikrein binding protein competes with or binds the same epitope as X67-D03 and, e.g., does not bind pre-plasma kallikrein.

In some embodiments, the plasma kallikrein binding protein competes with or binds to the same site as X101-A01 and, e.g., does not bind pre-plasma kallikrein.

In some embodiments, the plasma kallikrein binding protein competes with or binds to the same site as M162-A04 and, e.g., does not bind pre-plasma kallikrein.

In some embodiments, the plasma kallikrein binding protein competes with or binds to the same site as X115-F02 and, e.g., does not bind pre-plasma kallikrein.

In some embodiments, the plasma kallikrein binding protein competes with or binds to the same site as X124-G01 and, e.g., does not bind pre-plasma kallikrein.

In some embodiments, the plasma kallikrein binding protein competes with or binds to the same site as X63-G06 and, e.g., does not bind pre-plasma kallikrein.

In some embodiments, the plasma kallikrein binding protein does not bind prekallikrein (e.g., human prekallikrein and/or mouse prekallikrein), but binds to the active form of plasma kallikrein (e.g., human plasma kallikrein and/or mouse plasma kallikrein).

In certain embodiments, the protein binds at or near the active site of the catalytic domain of plasma kallikrein, or a fragment thereof, or binds an epitope that overlaps with the active site of plasma kallikrein and, e.g., does not bind pre-plasma kallikrein.

In some embodiments, the protein binds to one or more amino acids that form the catalytic triad of plasma kallikrein: His434, Asp483, and/or Ser578 (numbering based on the human sequence) and, e.g., does not bind pre-plasma kallikrein.

In some embodiments, the protein binds to one or more amino acids of: Ser479, Tyr563, and/or Asp585 (numbering based on the human sequence) and, e.g., does not bind pre-plasma kallikrein.

In some embodiments, the plasma kallikrein binding protein binds one or more amino acids of: Arg551, Gln553, Tyr555, Thr558, and/or Arg560 (numbering based on the human kallikrein sequence). In other embodiments, the plasma kallikrein binding protein binds two, three, four or five (i.e., all) amino acids of: Arg551, Gln553, Tyr555, Thr558, and/or Arg560 (numbering based on the human sequence) and, e.g., does not bind pre-plasma kallikrein.

In some embodiments, the plasma kallikrein binding protein binds one or more amino acids of: S478, N481, S525, and K526 (numbering based on the human kallikrein sequence). In other embodiments, the plasma kallikrein binding protein binds two, three or four (i.e., all) amino acids of: S478, N481, S525, and K526 (numbering based on the human kallikrein sequence).

In some embodiments, the plasma kallikrein binding protein decreases Factor XIIa and/or bradykinin production by greater than about 5%, about 10%, about 15%, about 20%, about 25%, about 30%, about 35%, about 40%, about 45%, about 50%, about 55%, about 60%, about 65%, about 70%, about 75%, about 80%, about 85%, about 90%, or about 95%

as compared to a standard, e.g., the Factor XIIa and/or bradykinin production under the same conditions but in the absence of the protein.

In some embodiments, the plasma kallikrein binding protein has an apparent inhibition constant ($K_{i,app}$) of less than 1000, 500, 100, 10, 1, 0.5 or 0.2 nM.

In one embodiment, the HC and LC variable domain sequences are components of the same polypeptide chain.

In another embodiment, the HC and LC variable domain sequences are components of different polypeptide chains. For example, the plasma kallikrein binding protein is an IgG, e.g., IgG1, IgG2, IgG3, or IgG4. The plasma kallikrein binding protein can be a soluble Fab (sFab).

In some embodiments, the plasma kallikrein binding protein has a serum residence time of 1 week, 2 weeks, 3 weeks, 4 weeks, 5 weeks or more, in vivo, e.g., in humans. In one embodiment, the plasma kallikrein binding protein is an IgG, e.g., an IgG1, IgG2, IgG3 or IgG4, that has a serum residence time of 1 week, 2 weeks, 3 weeks, 4 weeks, 5 weeks or more in vivo, e.g., in humans.

In some embodiments, the plasma kallikrein binding protein is physically associated with a moiety that improves serum residence time, e.g., a moiety described herein. In one embodiment, the plasma kallikrein binding protein is modified to include, e.g., PEGylation, fusion to serum albumin (e.g., human serum albumin), conjugation to human serum albumin, HESylation (HESylation utilises hydroxyethyl starch ("HES") derivatives linked to drug substances in order to modify the drug characteristics or fusion to a unstructured recombinant polymer (URPs).

In other embodiments, the plasma kallikrein binding protein includes a Fab2', scFv, minibody, scFv::Fc fusion, Fab::HSA fusion, HSA::Fab fusion, Fab::HSA::Fab fusion, or other molecule that comprises the antigen combining site of one of the binding proteins herein. The VH and VL regions of these Fabs can be provided as IgG, Fab, Fab2, Fab2', scFv, PEGylated Fab, PEGylated scFv, PEGylated Fab2, VH::CH1::HSA+LC, HSA::VH::CH1+LC, LC::HSA+VH::CH1, HSA::LC+VH::CH1, or other appropriate construction.

In one embodiment, the plasma kallikrein binding protein is a human or humanized antibody or is non-immunogenic in a human. For example, the protein includes one or more human antibody framework regions, e.g., all human framework regions.

In one embodiment, the plasma kallikrein binding protein includes a human Fc domain, or an Fc domain that is at least 95, 96, 97, 98, or 99% identical to a human Fc domain.

In one embodiment, the plasma kallikrein binding protein is a primate or primatized antibody or is non-immunogenic in a human. For example, the protein includes one or more primate antibody framework regions, e.g., all primate framework regions.

In one embodiment, the plasma kallikrein binding protein includes a primate Fc domain, or an Fc domain that is at least 95, 96, 97, 98, or 99% identical to a primate Fc domain. "Primate" includes humans (*Homo sapiens*), chimpanzees (*Pan troglodytes* and *Pan paniscus* (bonobos)), gorillas (*Gorilla gorilla*), gibons, monkeys, lemurs, aye-ayes (*Daubentonia madagascariensis*), and tarsiers.

In one embodiment, the plasma kallikrein binding protein includes human framework regions, or framework regions that are at least 95, 96, 97, 98, or 99% identical to human framework regions.

In certain embodiments, the plasma kallikrein binding protein includes no sequences from mice or rabbits (e.g., is not a murine or rabbit antibody).

In certain embodiments, the plasma kallikrein binding protein is capable of binding to a cell or tissue, e.g., that expresses plasma kallikrein.

In one embodiment, the plasma kallikrein binding protein is physically associated with a nanoparticle, and can be used to guide a nanoparticle to a cell or tissue expressing plasma kallikrein.

In some aspects, the disclosure features an isolated protein (e.g., antibody, e.g., human antibody) that binds the same epitope or competes for binding with a kallikrein binding protein described herein.

In some embodiments, the protein binds the same epitope or competes for binding with a protein (e.g., epi-Kal2) and/or a small molecule (e.g., AEBSF) described herein.

In some embodiments, the isolated protein comprises a heavy chain immunoglobulin variable domain sequence and a light chain immunoglobulin variable domain sequence, wherein:

the heavy chain immunoglobulin variable domain sequence comprises one, two, or three (e.g., three) CDR regions from the heavy chain variable domain of a protein described herein, and/or

the light chain immunoglobulin variable domain sequence comprises one, two, or three (e.g., three) CDR regions from the light chain variable domain of a protein described herein, wherein the protein binds to plasma kallikrein.

In some embodiments, the heavy chain immunoglobulin variable domain sequence comprises one, two, or three (e.g., three) CDR regions from the heavy chain variable domain of M162-A04, M199-A08, M160-G12, M142-H08, X63-G06, X101-A01, X81-B01, X67-D03, X67-G04, X115-B07, X115-D05, X115-E09, X115-H06, X115-A03, X115-D01, X115-F02, X124-G01, X115-G04, M29-D09, M145-D11, M06-D09 and M35-G04.

the light chain immunoglobulin variable domain sequence comprises one, two, or three (e.g., three) CDR regions from the light chain variable domain of M162-A04, M199-A08, M160-G12, M142-H08, X63-G06, X101-A01, X81-B01, X67-D03, X67-G04, X115-B07, X115-D05, X115-E09, X115-H06, X115-A03, X115-D01, X115-F02, X124-G01, X115-G04, M29-D09, M145-D11, M06-D09 and M35-G04 (respectively).

In some embodiments, the protein inhibits plasma kallikrein (e.g., human plasma kallikrein and/or mouse plasma kallikrein).

In some embodiments, the one, two, or three (e.g., three) CDR regions from the heavy chain variable domain are from X81-B01 and/or the one, two, or three (e.g., three) CDR regions from the light chain variable domain are from X81-B01.

In some embodiments, the one, two, or three (e.g., three) CDR regions from the heavy chain variable domain are from X67-D03 and/or the one, two, or three (e.g., three) CDR regions from the light chain variable domain are from X67-D03.

In some embodiments, the one, two, or three (e.g., three) CDR regions from the heavy chain variable domain are from X63-G06 and/or the one, two, or three (e.g., three) CDR regions from the light chain variable domain are from X63-G06.

In some embodiments, the one, two, or three (e.g., three) CDR regions from the heavy chain variable domain are from M162-A04 and/or the one, two, or three (e.g., three) CDR regions from the light chain variable domain are from MJ162-A04.

In some embodiments, the one, two, or three (e.g., three) CDR regions from the heavy chain variable domain are from

X115-F02 and/or the one, two, or three (e.g., three) CDR regions from the light chain variable domain are from X115-F02.

In some embodiments, the one, two, or three (e.g., three) CDR regions from the heavy chain variable domain are from X124-G01 and/or the one, two, or three (e.g., three) CDR regions from the light chain variable domain are from X124-G01.

In some embodiments, the heavy chain immunoglobulin variable domain sequence comprises the heavy chain variable domain of a protein described herein, and/or the light chain immunoglobulin variable domain sequence comprises the light chain variable domain of a protein described herein.

In some embodiments, the heavy chain immunoglobulin variable domain sequence comprises the heavy chain variable domain of M162-A04, M199-A08, M160-G12, M142-H08, X63-G06, X101-A01, X81-B01, X67-D03, X67-G04, X115-B07, X115-D05, X115-E09, X115-H06, X115-A03, X115-D01, X115-F02, X124-G01, X115-G04, M29-D09, M145-D11, M06-D09 and M35-G04, and/or the light chain immunoglobulin variable domain sequence comprises the light chain variable domain of M162-A04, M199-A08, M160-G12, M142-H08, X63-G06, X101-A01, X81-B01, X67-D03, X67-G04, X115-B07, X115-D05, X115-E09, X115-H06, X115-A03, X115-D01, X115-F02, X124-G01, X115-G04, M29-D09, M145-D11, M06-D09 and M35-G04 (respectively).

In some embodiments, the heavy chain immunoglobulin variable domain sequence comprises the heavy chain variable domain of X81-B01, and/or the light chain immunoglobulin variable domain sequence comprises the light chain variable domain of X81-B01.

In some embodiments, the heavy chain immunoglobulin variable domain sequence comprises the heavy chain variable domain of X67-D03, and/or the light chain immunoglobulin variable domain sequence comprises the light chain variable domain of X67-D03.

In some embodiments, the protein comprises the heavy chain of a protein described herein, and/or the light chain of a protein described herein.

In some embodiments, the protein comprises the heavy chain of M162-A04, M199-A08, M160-G12, M142-H08, X63-G06, X101-A01, X81-B01, X67-D03, X67-G04, X115-B07, X115-D05, X115-E09, X115-H06, X115-A03, X115-D01, X115-F02, X124-G01, X115-G04, M29-D09, M145-D11, M06-D09 and M35-G04.

and/or the light chain of M162-A04, M199-A08, M160-G12, M142-H08, X63-G06, X101-A01, X81-B01, X67-D03, X67-G04, X115-B07, X115-D05, X115-E09, X115-H06, X115-A03, X115-D01, X115-F02, X124-G01, X115-G04, M29-D09, M145-D11, M06-D09 and M35-G04 (respectively).

In some embodiments, the protein comprises the heavy chain of X81-B01, and/or the light chain of X81-B01.

In some embodiments, the protein comprises the heavy chain of X67-D03, and/or the light chain of X67-D03.

In some embodiments, the protein comprises the heavy chain of M162-A04, and/or the light chain of M162-A04.

In some embodiments, the protein comprises the heavy chain of X115-F02, and/or the light chain of X115-F02.

In some embodiments, the protein comprises the heavy chain of X124-G01, and/or the light chain of X124-G01.

In some embodiments, the plasma kallikrein binding protein does not bind prekallikrein (e.g., human prekallikrein and/or mouse prekallikrein), but binds to the active form of plasma kallikrein (e.g., human plasma kallikrein and/or mouse plasma kallikrein).

In some embodiments, the plasma kallikrein binding protein decreases Factor XIIa and/or bradykinin production by greater than about 5%, about 10%, about 15%, about 20%, about 25%, about 30%, about 35%, about 40%, about 45%, about 50%, about 55%, about 60%, about 65%, about 70%, about 75%, about 80%, about 85%, about 90%, or about 95% as compared to a standard, e.g., the Factor XIIa and/or bradykinin production under the same conditions but in the absence of the protein.

In some embodiments, the protein includes one or more of the following characteristics: (a) a human CDR or human framework region; (b) the HC immunoglobulin variable domain sequence comprises one or more (e.g., 1, 2, or 3) CDRs that are at least 85, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, or 100% identical to a CDR of a HC variable domain described herein; (c) the LC immunoglobulin variable domain sequence comprises one or more (e.g., 1, 2, or 3) CDRs that are at least 85, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, or 100% identical to a CDR of a LC variable domain described herein; (d) the LC immunoglobulin variable domain sequence is at least 85, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, or 100% identical to a LC variable domain described herein (e.g., overall or in framework regions or CDRs); (e) the HC immunoglobulin variable domain sequence is at least 85, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, or 100% identical to a HC variable domain described herein (e.g., overall or in framework regions or CDRs); (f) the protein binds an epitope bound by a protein described herein, or competes for binding with a protein described herein; (g) a primate CDR or primate framework region; (h) the HC immunoglobulin variable domain sequence comprises a CDR1 that differs by at least one amino acid but by no more than 2 or 3 amino acids from the CDR1 of a HC variable domain described herein; (i) the HC immunoglobulin variable domain sequence comprises a CDR2 that differs by at least one amino acid but by no more than 2, 3, 4, 5, 6, 7, or 8 amino acids from the CDR2 of a HC variable domain described herein; (j) the HC immunoglobulin variable domain sequence comprises a CDR3 that differs by at least one amino acid but by no more than 2, 3, 4, 5, or 6 amino acids from the CDR3 of a HC variable domain described herein; (k) the LC immunoglobulin variable domain sequence comprises a CDR1 that differs by at least one amino acid but by no more than 2, 3, 4, or 5 amino acids from the CDR1 of a LC variable domain described herein; (l) the LC immunoglobulin variable domain sequence comprises a CDR2 that differs by at least one amino acid but by no more than 2, 3, or 4 amino acids from the CDR2 of a LC variable domain described herein; (m) the LC immunoglobulin variable domain sequence comprises a CDR3 that differs by at least one amino acid but by no more than 2, 3, 4, or 5 amino acids from the CDR3 of a LC variable domain described herein; (n) the LC immunoglobulin variable domain sequence differs by at least one amino acid but by no more than 2, 3, 4, 5, 6, 7, 8, 9, or 10 amino acids from a LC variable domain described herein (e.g., overall or in framework regions or CDRs); and (o) the HC immunoglobulin variable domain sequence differs by at least one amino acid but by no more than 2, 3, 4, 5, 6, 7, 8, 9, or 10 amino acids from a HC variable domain described herein (e.g., overall or in framework regions or CDRs).

In some embodiments, the protein has an apparent inhibition constant ($K_{i,app}$) of less than 1000, 500, 100, 10, 1, 0.5 or 0.2 nM.

In some embodiments, the antibody does not bind prekallikrein (e.g., human prekallikrein and/or mouse prekallikrein), but binds to the active form of plasma kallikrein (e.g., human plasma kallikrein and/or mouse plasma kallikrein).

In a preferred embodiment, the protein is an antibody (e.g., a human antibody) having the light and heavy chains of antibodies selected from the group consisting of M162-A04, M199-A08, M160-G12, M142-H08, X63-G06, X101-A01, X81-B01, X67-D03, X67-G04, X115-B07, X115-D05, X115-E09, X115-H06, X115-A03, X115-D01, X115-F02, X124-G01, X115-G04, M29-D09, M145-D11, M06-D09 and M35-G04.

In a preferred embodiment, the protein is an antibody (e.g., a human antibody) having the heavy chain of an antibody selected from the group consisting of: M162-A04, M199-A08, M160-G12, M142-H08, X63-G06, X101-A01, X81-B01, X67-D03, X67-G04, X115-B07, X115-D05, X115-E09, X115-H06, X115-A03, X115-D01, X115-F02, X124-G01, X115-G04, M29-D09, M145-D11, M06-D09 and M35-G04.

In a preferred embodiment, the protein is an antibody (e.g., a human antibody) having the light chain of an antibody selected from the group consisting of: M162-A04, M199-A08, M160-G12, M142-H08, X63-G06, X101-A01, X81-B01, X67-D03, X67-G04, X115-B07, X115-D05, X115-E09, X115-H06, X115-A03, X115-D01, X115-F02, X124-G01, X115-G04, M29-D09, M145-D11, M06-D09 and M35-G04.

In a preferred embodiment, the protein is an antibody (e.g., a human antibody) having light and heavy antibody variable regions of an antibody selected from the group consisting of M162-A04, M199-A08, M160-G12, M142-H08, X63-G06, X101-A01, X81-B01, X67-D03, X67-G04, X115-B07, X115-D05, X115-E09, X115-H06, X115-A03, X115-D01, X115-F02, X124-G01, X115-G04, M29-D09, M145-D11, M06-D09 and M35-G04.

In a preferred embodiment, the protein is an antibody (e.g., a human antibody) having a heavy chain antibody variable region of an antibody selected from the group consisting of: M162-A04, M199-A08, M160-G12, M142-H08, X63-G06, X101-A01, X81-B01, X67-D03, X67-G04, X115-B07, X115-D05, X115-E09, X115-H06, X115-A03, X115-D01, X115-F02, X124-G01, X115-G04, M29-D09, M145-D11, M06-D09 and M35-G04.

In a preferred embodiment, the protein is an antibody (e.g., a human antibody) having a light chain antibody variable region of an antibody selected from the group consisting of: M162-A04, M199-A08, M160-G12, M142-H08, X63-G06, X101-A01, X81-B01, X67-D03, X67-G04, X115-B07, X115-D05, X115-E09, X115-H06, X115-A03, X115-D01, X115-F02, X124-G01, X115-G04, M29-D09, M145-D11, M06-D09 and M35-G04.

In a preferred embodiment, the protein is an antibody (e.g., a human antibody) having one or more (e.g., 1, 2, or 3) heavy chain CDRs selected from the corresponding CDRs of the group of heavy chains consisting of M162-A04, M199-A08, M160-G12, M142-H08, X63-G06, X101-A01, X81-B01, X67-D03, X67-G04, X115-B07, X115-D05, X115-E09, X115-H06, X115-A03, X115-D01, X115-F02, X124-G01, X115-G04, M29-D09, M145-D11, M06-D09 and M35-G04.

In a preferred embodiment, the protein is an antibody (e.g., a human antibody) having one or more (e.g., 1, 2, or 3) light chain CDRs selected from the corresponding CDRs of the group of light chains consisting of M162-A04, M199-A08, M160-G12, M142-H08, X63-G06, X101-A01, X81-B01, X67-D03, X67-G04, X115-B07, X115-D05, X115-E09, X115-H06, X115-A03, X115-D01, X115-F02, X124-G01, X115-G04, M29-D09, M145-D11, M06-D09 and M35-G04.

In a preferred embodiment, the protein is an antibody (e.g., a human antibody) having one or more (e.g., 1, 2, or 3) heavy chain CDRs selected from the corresponding CDRs of the group of heavy chains consisting of M162-A04, M199-A08, M160-G12, M142-H08, X63-G06, X101-A01, X81-B01, X67-D03, X67-G04, X115-B07, X115-D05, X115-E09, X115-H06, X115-A03, X115-D01, X115-F02, X124-G01, X115-G04, M29-D09, M145-D11, M06-D09 and M35-G04.

and one or more (e.g., 1, 2, or 3) light chain CDRs selected from the corresponding CDRs of the group of light chains consisting of M162-A04, M199-A08, M160-G12, M142-H08, X63-G06, X101-A01, X81-B01, X67-D03, X67-G04, X115-B07, X115-D05, X115-E09, X115-H06, X115-A03, X115-D01, X115-F02, X124-G01, X115-G04, M29-D09, M145-D11, M06-D09 and M35-G04 (respectively).

In a preferred embodiment, the protein is an antibody (e.g., a human antibody) having the light and heavy chains of X81-B01.

In a preferred embodiment, the protein is an antibody (e.g., a human antibody) having the heavy chain of X81-B01.

In a preferred embodiment, the protein is an antibody (e.g., a human antibody) having the light chain of X81-B01.

In a preferred embodiment, the protein is an antibody (e.g., a human antibody) having light and heavy antibody variable regions of an antibody selected from X81-B01.

In a preferred embodiment, the protein is an antibody (e.g., a human antibody) having a heavy chain antibody variable region of X81-B01.

In a preferred embodiment, the protein is an antibody (e.g., a human antibody) having a light chain antibody variable region of X81-B01.

In a preferred embodiment, the protein is an antibody (e.g., a human antibody) having one or more (e.g., 1, 2, or 3) heavy chain CDRs from the corresponding CDRs of the heavy chain of X81-B01.

In a preferred embodiment, the protein is an antibody (e.g., a human antibody) having one or more (e.g., 1, 2, or 3) light chain CDRs from the corresponding CDRs of the light chain of X81-B01.

In a preferred embodiment, the protein is an antibody (e.g., a human antibody) having one or more (e.g., 1, 2, or 3) heavy chain CDRs from the heavy chain of X81-B01 and one or more (e.g., 1, 2, or 3) light chain CDRs from the corresponding CDRs of the light chain of X81-B01.

In a preferred embodiment, the protein is an antibody (e.g., a human antibody) having the light and heavy chains of X67-D03.

In a preferred embodiment, the protein is an antibody (e.g., a human antibody) having the heavy chain of X67-D03.

In a preferred embodiment, the protein is an antibody (e.g., a human antibody) having the light chain of X67-D03.

In a preferred embodiment, the protein is an antibody (e.g., a human antibody) having light and heavy antibody variable regions of an antibody selected from X67-D03.

In a preferred embodiment, the protein is an antibody (e.g., a human antibody) having a heavy chain antibody variable region of X67-D03.

In a preferred embodiment, the protein is an antibody (e.g., a human antibody) having a light chain antibody variable region of X67-D03.

In a preferred embodiment, the protein is an antibody (e.g., a human antibody) having one or more (e.g., 1, 2, or 3) heavy chain CDRs from the corresponding CDRs of the heavy chain of X67-D03.

In a preferred embodiment, the protein is an antibody (e.g., a human antibody) having one or more (e.g., 1, 2, or 3) light chain CDRs from the corresponding CDRs of the light chain of X67-D03.

In a preferred embodiment, the protein is an antibody (e.g., a human antibody) having one or more (e.g., 1, 2, or 3) heavy chain CDRs from the heavy chain of X67-D03 and one or more (e.g., 1, 2, or 3) light chain CDRs from the corresponding CDRs of the light chain of X67-D03.

In a preferred embodiment, the protein is an antibody (e.g., a human antibody) having the light and heavy chains of X124-G01 or X115-F02.

In a preferred embodiment, the protein is an antibody (e.g., a human antibody) having the heavy chain of X124-G01 or X115-F02.

In a preferred embodiment, the protein is an antibody (e.g., a human antibody) having the light chain of X124-G01 or X115-F02.

In a preferred embodiment, the protein is an antibody (e.g., a human antibody) having light and heavy antibody variable regions of an antibody selected from X124-G01 or X115-F02.

In a preferred embodiment, the protein is an antibody (e.g., a human antibody) having a heavy chain antibody variable region of X124-G01 or X115-F02.

In a preferred embodiment, the protein is an antibody (e.g., a human antibody) having a light chain antibody variable region of X124-G01 or X115-F02.

In a preferred embodiment, the protein is an antibody (e.g., a human antibody) having one or more (e.g., 1, 2, or 3) heavy chain CDRs from the corresponding CDRs of the heavy chain of X124-G01 or X115-F02.

In a preferred embodiment, the protein is an antibody (e.g., a human antibody) having one or more (e.g., 1, 2, or 3) light chain CDRs from the corresponding CDRs of the light chain of X124-G01 or X115-F02.

In a preferred embodiment, the protein is an antibody (e.g., a human antibody) having one or more (e.g., 1, 2, or 3) heavy chain CDRs from the heavy chain of X124-G01 or X115-F02 and one or more (e.g., 1, 2, or 3) light chain CDRs from the corresponding CDRs of the light chain of X124-G01 or X115-F02.

In one embodiment, the HC and LC variable domain sequences are components of the same polypeptide chain.

In some embodiments, the plasma kallikrein binding protein has a serum residence time of 1 week, 2 weeks, 3 weeks, 4 weeks, 5 weeks or more, in vivo, e.g., in humans. In one embodiment, the plasma kallikrein binding protein is an IgG, e.g., an IgG1, IgG2, IgG3 or IgG4, that has a serum residence time of 1 week, 2 weeks, 3 weeks, 4 weeks, 5 weeks or more in vivo, e.g., in humans.

In some embodiments, the plasma kallikrein binding protein is physically associated with a moiety that improves serum residence time, e.g., a moiety described herein. In one embodiment, the plasma kallikrein binding protein is modified to include, e.g., PEGylation, fusion to serum albumin (e.g., human serum albumin), conjugation to human serum albumin, HESylation (HESylation utilises hydroxyethyl starch ("HES") derivatives linked to drug substances in order to modify the drug characteristics or fusion to a unstructured recombinant polymer (URPs).

In another embodiment, the HC and LC variable domain sequences are components of different polypeptide chains. For example, the protein is an IgG, e.g., IgG1, IgG2, IgG3, or IgG4. The protein can be a soluble Fab (sFab).

In other embodiments, the protein includes a Fab2', scFv, minibody, scFv::Fc fusion, Fab::HSA fusion, HSA::Fab fusion, Fab::HSA::Fab fusion, or other molecule that comprises the antigen combining site of one of the binding proteins herein. The VH and VL regions of these Fabs can be provided as IgG, Fab, Fab2, Fab2', scFv, PEGylated Fab, PEGylated scFv, PEGylated Fab2, VH::CH1::HSA+LC, HSA::VH::CH1+LC, LC::HSA+VH::CH1, HSA::LC+VH::CH1, or other appropriate construction.

In one embodiment, the protein is a human or humanized antibody or is non-immunogenic in a human. For example, the protein includes one or more human antibody framework regions, e.g., all human framework regions.

In one embodiment, the protein includes a human Fc domain, or an Fc domain that is at least 95, 96, 97, 98, or 99% identical to a human Fc domain.

In one embodiment, the protein is a primate or primatized antibody or is non-immunogenic in a human. For example, the protein includes one or more primate antibody framework regions, e.g., all primate framework regions.

In one embodiment, the protein includes a primate Fc domain, or an Fc domain that is at least 95, 96, 97, 98, or 99% identical to a primate Fc domain. "Primate" includes humans (*Homo sapiens*), chimpanzees (*Pan troglodytes* and *Pan paniscus* (bonobos)), gorillas (*Gorilla gorilla*), gibons, monkeys, lemurs, aye-ayes (*Daubentonia madagascariensis*), and tarsiers.

In one embodiment, the protein includes human framework regions, or framework regions that are at least 95, 96, 97, 98, or 99% identical to human framework regions.

In certain embodiments, the protein includes no sequences from mice or rabbits (e.g., is not a murine or rabbit antibody).

In certain embodiments, the protein is capable of binding to a cell or tissue, e.g., that expresses plasma kallikrein.

In one embodiment, protein is physically associated with a nanoparticle, and can be used to guide a nanoparticle to a cell or tissue expressing plasma kallikrein.

In some aspects, the disclosure features a pharmaceutical composition comprising a kallikrein binding protein described herein, e.g., including a pharmaceutically acceptable carrier. In some embodiments, the composition can be at least 10, 20, 30, 50, 75, 85, 90, 95, 98, 99, or 99.9% free of other protein species. In one embodiment, the pharmaceutical composition can be at least 10, 20, 30, 50, 75, 85, 90, 95, 98, 99, or 99.9% free of fragments of the binding protein that do not binding plasma kallikrein (e.g., human plasma kallikrein) or bind plasma kallikrein (e.g., human plasma kallikrein with a Ki, app of 5000 nM or greater.

In some aspects, the disclosure features a method of treating or preventing a plasma kallikrein associated disorder in a subject, the method comprising:

administering an isolated protein (e.g., antibody, e.g., human antibody) that binds plasma kallikrein (e.g., human plasma kallikrein and/or mouse plasma kallikrein) and, e.g., does not bind prekallikrein (e.g., human prekallikrein and/or mouse prekallikrein) to the subject,

In some embodiments, the protein binds the same epitope or competes for binding with a protein (e.g., epi-Kal2) and/or a small molecule (e.g., AEBSF) described herein.

In some embodiments, the protein binds the same epitope or competes for binding with a kallikrein binding protein described herein.

In some embodiments, the plasma kallikrein associated disorder is selected from the group consisting of rheumatoid arthritis, gout, intestinal bowel disease, oral mucositis, neuropathic pain, inflammatory pain, spinal stenosis-degenerative spine disease, arterial or venous thrombosis, post operative ileus, aortic aneurysm, osteoarthritis, vasculitis, edema, hereditary angioedema, cerebral edema, pulmonary embolism, stroke, clotting on ventricular assistance devices or stents, head trauma or peri-tumor brain edema, sepsis, acute middle cerebral artery (MCA) ischemic event (stroke), restenosis (e.g., after angioplasty), systemic lupus erythematosis nephritis, and burn injury. In some embodiments, the plasma kallikrein binding protein reduces abberent clotting associated with the contact activation system (i.e., intrinsic activation system) by at least 10% as measured by e.g., an APTT clotting assay. In other embodiments, the plasma kallikrein binding protein reduces abberent clotting associated with the contact activation system by at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, at least 99%, or even 100% (i.e., no detectable abberent clotting).

In some embodiments, the plasma kallikrein binding protein is administered in combination with another treatment for the disorder.

In some embodiments, the protein described herein is selected from the group consisting of M162-A04, M199-A08, M160-G12, M142-H08, X63-G06, X101-A01, X81-B01, X67-D03, X67-G04, X115-B07, X115-D05, X115-E09, X115-H06, X115-A03, X115-D01, X115-F02, X124-G01, X115-G04, M29-D09, M145-D11, M06-D09 and M35-G04.

In some embodiments, the plasma kallikrein binding protein competes with or binds the same epitope as X81-B01.

In some embodiments, the plasma kallikrein binding protein competes with or binds the same epitope as X67-D03.

In some embodiments, the plasma kallikrein binding protein competes with or binds to the same epitope as M162-A04 or X115-F02.

In some embodiments, the plasma kallikrein binding protein does not bind prekallikrein (e.g., human prekallikrein and/or mouse prekallikrein), but binds to the active form of plasma kallikrein (e.g., human plasma kallikrein and/or mouse plasma kallikrein).

In certain embodiments, the protein binds at or near the active site of the catalytic domain of plasma kallikrein, or a fragment thereof, or binds an epitope that overlaps with the active site of plasma kallikrein.

In some embodiments, the protein binds to one or more amino acids that form the catalytic triad of plasma kallikrein: His434, Asp483, and/or Ser578 (numbering based on the human sequence).

In some embodiments, the protein binds to one or more amino acids of Ser479, Tyr563, and/or Asp585 (numbering based on the human sequence).

In other embodiments, the protein binds to one or more amino acids of Arg551, Gln553, Tyr555, Thr558, and/or Arg560 (numbering based on the human sequence). In some embodiments, the plasma kallikrein binding protein binds one or more amino acids of: S478, N481, S525, and K526 (numbering based on the human kallikrein sequence).

In some embodiments, the plasma kallikrein binding protein decreases Factor XIIa and/or bradykinin production by greater than about 5%, about 10%, about 15%, about 20%, about 25%, about 30%, about 35%, about 40%, about 45%, about 50%, about 55%, about 60%, about 65%, about 70%, about 75%, about 80%, about 85%, about 90%, or about 95% as compared to a standard, e.g., the Factor XIIa and/or bradykinin production under the same conditions but in the absence of the protein.

In some embodiments, the plasma kallikrein binding protein has an apparent inhibition constant ($K_{i,app}$) of less than 1000, 500, 100, 10, 5, 1, 0.5, or 0.2 nM.

In one embodiment, the HC and LC variable domain sequences are components of the same polypeptide chain.

In some embodiments, the plasma kallikrein binding protein has a serum residence time of 1 week, 2 weeks, 3 weeks, 4 weeks, 5 weeks or more, in vivo, e.g., in humans. In one embodiment, the plasma kallikrein binding protein is an IgG, e.g., an IgG1, IgG2, IgG3 or IgG4, that has a serum residence time of 1 week, 2 weeks, 3 weeks, 4 weeks, 5 weeks or more in vivo, e.g., in humans.

In some embodiments, the plasma kallikrein binding protein is physically associated with a moiety that improves serum residence time, e.g., a moiety described herein.

In another embodiment, the HC and LC variable domain sequences are components of different polypeptide chains. For example, the plasma kallikrein binding protein is an IgG, e.g., IgG1, IgG2, IgG3, or IgG4. The plasma kallikrein binding protein can be a soluble Fab (sFab).

In other implementations the plasma kallikrein binding protein includes a Fab2', scFv, minibody, scFv::Fc fusion, Fab::HSA fusion, HSA::Fab fusion, Fab::HSA::Fab fusion, or other molecule that comprises the antigen combining site of one of the binding proteins herein. The VH and VL regions of these Fabs can be provided as IgG, Fab, Fab2, Fab2', scFv, PEGylated Fab, PEGylated scFv, PEGylated Fab2, VH::CH1::HSA+LC, HSA::VH::CH1+LC, LC::HSA+VH::CH1, HSA::LC+VH::CH1, or other appropriate construction.

In one embodiment, the plasma kallikrein binding protein is a human or humanized antibody or is non-immunogenic in a human. For example, the protein includes one or more human antibody framework regions, e.g., all human framework regions.

In one embodiment, the plasma kallikrein binding protein includes a human Fc domain, or an Fc domain that is at least 95, 96, 97, 98, or 99% identical to a human Fc domain.

In one embodiment, the plasma kallikrein binding protein is a primate or primatized antibody or is non-immunogenic in a human. For example, the protein includes one or more primate antibody framework regions, e.g., all primate framework regions.

In one embodiment, the plasma kallikrein binding protein includes a primate Fc domain, or an Fc domain that is at least 95, 96, 97, 98, or 99% identical to a primate Fc domain. "Primate" includes humans (*Homo sapiens*), chimpanzees (*Pan troglodytes* and *Pan paniscus* (bonobos)), gorillas (*Gorilla gorilla*), gibons, monkeys, lemurs, aye-ayes (*Daubentonia madagascariensis*), and tarsiers.

In one embodiment, the plasma kallikrein binding protein includes human framework regions, or framework regions that are at least 95, 96, 97, 98, or 99% identical to human framework regions.

In certain embodiments, the plasma kallikrein binding protein includes no sequences from mice or rabbits (e.g., is not a murine or rabbit antibody).

In certain embodiments, the plasma kallikrein binding protein is capable of binding to a cell or tissue, e.g., that expresses plasma kallikrein.

In one embodiment, the plasma kallikrein binding protein is physically associated with a nanoparticle, and can be used to guide a nanoparticle to a cell or tissue expressing plasma kallikrein.

A method of treating or preventing a plasma kallikrein associated disorder in a subject, the method comprising:

administering an isolated protein (e.g., antibody, e.g., human antibody) comprising a heavy chain immunoglobulin variable domain sequence and a light chain immunoglobulin variable domain sequence to the subject, wherein:

the heavy chain immunoglobulin variable domain sequence comprises one, two, or three (e.g., three) CDR regions from the heavy chain variable domain of a protein described herein, and/or

the light chain immunoglobulin variable domain sequence comprises one, two, or three (e.g., three) CDR regions from the light chain variable domain of a protein described herein, wherein the protein binds to plasma kallikrein (e.g., human plasma kallikrein and/or mouse plasma kallikrein).

In some embodiments, the plasma kallikrein associated disorder is selected from the group consisting of rheumatoid arthritis, gout, intestinal bowel disease, oral mucositis, neuropathic pain, inflammatory pain, spinal stenosis-degenerative spine disease, arterial or venous thrombosis, post operative ileus, aortic aneurysm, osteoarthritis, vasculitis, edema, hereditary angioedema, cerebral edema, pulmonary embolism, stroke, clotting of ventrical assistance devices or stents, head trauma or peri-tumor brain edema, sepsis, acute middle cerebral artery (MCA) ischemic event (stroke), restenosis (e.g., after angioplasty), systemic lupus erythematosis nephritis, and burn injury. In some embodiments, the plasma kallikrein binding protein reduces abberent clotting associated with the contact activation system (i.e., intrinsic activation system) by at least 10% as measured by e.g., an APTT clotting assay (e.g., by at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, at least 99%, or even 100% (i.e., no detectable abberent clotting)).

In some embodiments, the protein is administered in combination with another treatment for the disorder.

In some embodiments, the protein is administered in combination with a second agent selected from the group consisting of ecallantide, a C1 esterase inhibitor, aprotinin, a bradykinin B2 receptor inhibitor (e.g., icatibant).

In some embodiments, the heavy chain immunoglobulin variable domain sequence comprises one, two, or three (e.g., three) CDR regions from the heavy chain variable domain of M162-A04, M199-A08, M160-G12, M142-H08, X63-G06, X101-A01, X81-B01, X67-D03, X67-G04, X115-B07, X115-D05, X115-E09, X115-H06, X115-A03, X115-D01, X115-F02, X124-G01, X115-G04, M29-D09, M145-D11, M06-D09 and M35-G04, and/or the light chain immunoglobulin variable domain sequence comprises one, two, or three (e.g., three) CDR regions from the light chain variable domain of M162-A04, M199-A08, M160-G12, M142-H08, X63-G06, X101-A01, X81-B01, X67-D03, X67-G04, X115-B07, X115-D05, X115-E09, X115-H06, X115-A03, X115-D01, X115-F02, X124-G01, X115-G04, M29-D09, M145-D11, M06-D09 and M35-G04 (respectively).

In some embodiments, the protein inhibits plasma kallikrein.

In some embodiments, the one, two, or three (e.g., three) CDR regions from the heavy chain variable domain are from X81-B01 and/or the one, two, or three (e.g., three) CDR regions from the light chain variable domain are from X81-B01.

In some embodiments, the one, two, or three (e.g., three) CDR regions from the heavy chain variable domain are from X67-D03 and/or the one, two, or three (e.g., three) CDR regions from the light chain variable domain are from X67-D03.

In some embodiments, the one, two, or three (e.g., three) CDR regions from the heavy chain variable domain are from M162-A04 and/or the one, two, or three (e.g., three) CDR regions from the light chain variable domain are from M162-A04.

In some embodiments, the one, two, or three (e.g., three) CDR regions from the heavy chain variable domain are from X115-F02 and/or the one, two, or three (e.g., three) CDR regions from the light chain variable domain are from X115-F02.

In some embodiments, the heavy chain immunoglobulin variable domain sequence comprises the heavy chain variable domain of a protein described herein, and/or the light chain immunoglobulin variable domain sequence comprises the light chain variable domain of a protein described herein.

In some embodiments, the heavy chain immunoglobulin variable domain sequence comprises the heavy chain variable domain of M162-A04, M199-A08, M160-G12, M142-H08×63-G06, X101-A01, X81-B01, X67-D03, X67-G04, X115-B07, X115-D05, X115-E09, X115-H06, X115-A03, X115-D01, X115-F02, X124-G01, X115-G04, M29-D09, M145-D11, M06-D09 and M35-G04, and/or the light chain immunoglobulin variable domain sequence comprises the light chain variable domain of M162-A04, M199-A08, M160-G12, M142-H08, X63-G06, X101-A01, X81-B01, X67-D03, X67-G04, X115-B07, X115-D05, X115-E09, X115-H06, X115-A03, X115-D01, X115-F02, X124-G01, X115-G04, M29-D09, M145-D11, M06-D09 and M35-G04.

In some embodiments, the heavy chain immunoglobulin variable domain sequence comprises the heavy chain variable domain of X81-B01, and/or the light chain immunoglobulin variable domain sequence comprises the light chain variable domain of X81-B01.

In some embodiments, the heavy chain immunoglobulin variable domain sequence comprises the heavy chain variable domain of X67-D03, and/or the light chain immunoglobulin variable domain sequence comprises the light chain variable domain of X67-D03.

In some embodiments, the protein comprises the heavy chain of a protein described herein, and/or the light chain of a protein described herein.

In some embodiments, the protein comprises the heavy chain of M162-A04, M199-A08, M160-G12, M142-H08, X63-G06, X101-A01, X81-B01, X67-D03, X67-G04, X115-B07, X115-D05, X115-E09, X115-H06, X115-A03, X115-D01, X115-F02, X124-G01, X115-G04, M29-D09, M145-D11, M06-D09 and M35-G04, and/or the light chain of M162-A04, M199-A08, M160-G12, M142-H08, X63-G06, X101-A01, X81-B01, X67-D03, X67-G04, X115-B07, X115-D05, X115-E09, X115-H06, X115-A03, X115-D01, X115-F02, X124-G01, X115-G04, M29-D09, M145-D11, M06-D09 and M35-G04 (respectively).

In some embodiments, the protein comprises the heavy chain of X81-B01, and/or the light chain of X81-B01.

In some embodiments, the protein comprises the heavy chain of X67-D03, and/or the light chain of X67-D03.

In some embodiments, the protein comprises the heavy chain of M162-A04, and/or the light chain of M162-A04.

In some embodiments, the protein comprises the heavy chain of X115-F02 or X124-G01, and/or the light chain of X115-F02 or X124-G01.

In some embodiments, the plasma kallikrein binding protein does not bind prekallikrein (e.g., human prekallikrein and/or murine prekallikrein), but binds to the active form of plasma kallikrein (e.g., human plasma kallikrein and/or murine plasma kallikrein).

In some embodiments, the plasma kallikrein binding protein decreases Factor XIIa and/or bradykinin production by greater than about 5%, about 10%, about 15%, about 20%, about 25%, about 30%, about 35%, about 40%, about 45%, about 50%, about 55%, about 60%, about 65%, about 70%, about 75%, about 80%, about 85%, about 90%, or about 95% as compared to a standard, e.g., the Factor XIIa and/or bradykinin production under the same conditions but in the absence of the protein.

In some embodiments, the protein includes one or more of the following characteristics: (a) a human CDR or human framework region; (b) the HC immunoglobulin variable domain sequence comprises one or more (e.g., 1, 2, or 3) CDRs that are at least 85, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, or 100% identical to a CDR of a HC variable domain described herein; (c) the LC immunoglobulin variable domain sequence comprises one or more (e.g., 1, 2, or 3) CDRs that are at least 85, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, or 100% identical to a CDR of a LC variable domain described herein; (d) the LC immunoglobulin variable domain sequence is at least 85, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, or 100% identical to a LC variable domain described herein (e.g., overall or in framework regions or CDRs); (e) the HC immunoglobulin variable domain sequence is at least 85, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, or 100% identical to a HC variable domain described herein (e.g., overall or in framework regions or CDRs); (f) the protein binds an epitope bound by a protein described herein, or competes for binding with a protein described herein; and (g) a primate CDR or primate framework region.

In some embodiments, the protein has an apparent inhibition constant ($K_{i,app}$) of less than 1000, 500, 100, 10, 5, 1, 0.5 or 0.2 nM.

In some embodiments, the antibody does not bind prekallikrein (e.g., human prekallikrein), but binds to the active form of plasma kallikrein (e.g., human plasma kallikrein).

In a preferred embodiment, the protein is an antibody (e.g., a human antibody) having the light and heavy chains of antibodies selected from the group consisting of M162-A04, M199-A08, M160-G12, M142-H08, X63-G06, X101-A01, X81-B01, X67-D03, X67-G04, X115-B07, X115-D05, X115-E09, X115-H06, X115-A03, X115-D01, X115-F02, X124-G01, X115-G04, M29-D09, M145-D11, M06-D09 and M35-G04.

In a preferred embodiment, the protein is an antibody (e.g., a human antibody) having the heavy chain of an antibody selected from the group consisting of: M162-A04, M199-A08, M160-G12, M142-H08, X63-G06, X101-A01, X81-B01, X67-D03, X67-G04, X115-B07, X115-D05, X115-E09, X115-H06, X115-A03, X115-D01, X115-F02, X124-G01, X115-G04, M29-D09, M145-D11, M06-D09 and M35-G04.

In a preferred embodiment, the protein is an antibody (e.g., a human antibody) having the light chain of an antibody selected from the group consisting of: M162-A04, M199-A08, M160-G12, M142-H08, X63-G06, X101-A01, X81-B01, X67-D03, X67-G04, X115-B07, X115-D05, X115-E09, X115-H06, X115-A03, X115-D01, X115-F02, X124-G01, X115-G04, M29-D09, M145-D11, M06-D09 and M35-G04.

In a preferred embodiment, the protein is an antibody (e.g., a human antibody) having light and heavy antibody variable regions of an antibody selected from the group consisting of M162-A04, M199-A08, M160-G12, M142-H08, X63-G06, X101-A01, X81-B01, X67-D03, X67-G04, X115-B07, X115-D05, X115-E09, X115-H06, X115-A03, X115-D01, X115-F02, X124-G01, X115-G04, M29-D09, M145-D11, M06-D09 and M35-G04.

In a preferred embodiment, the protein is an antibody (e.g., a human antibody) having a heavy chain antibody variable region of an antibody selected from the group consisting of: M162-A04, M199-A08, M160-G12, M142-H08, X63-G06, X101-A01, X81-B01, X67-D03, X67-G04, X115-B07, X115-D05, X115-E09, X115-H06, X115-A03, X115-D01, X115-F02, X124-G01, X115-G04, M29-D09, M145-D11, M06-D09 and M35-G04.

In a preferred embodiment, the protein is an antibody (e.g., a human antibody) having a light chain antibody variable region of an antibody selected from the group consisting of: M162-A04, M199-A08, M160-G12, M142-H08, X63-G06, X101-A01, X81-B01, X67-D03, X67-G04, X115-B07, X115-D05, X115-E09, X115-H06, X115-A03, X115-D01, X115-F02, X124-G01, X115-G04, M29-D09, M145-D11, M06-D09 and M35-G04.

In a preferred embodiment, the protein is an antibody (e.g., a human antibody) having one or more (e.g., 1, 2, or 3) heavy chain CDRs selected from the corresponding CDRs of the group of heavy chains consisting of M162-A04, M199-A08, M160-G12, M142-H08, X63-G06, X101-A01, X81-B01, X67-D03, X67-G04, X115-B07, X115-D05, X115-E09, X115-H06, X115-A03, X115-D01, X115-F02, X124-G01, X115-G04, M29-D09, M145-D11, M06-D09 and M35-G04.

In a preferred embodiment, the protein is an antibody (e.g., a human antibody) having one or more (e.g., 1, 2, or 3) light chain CDRs selected from the corresponding CDRs of the group of light chains consisting of M162-A04, M199-A08, M160-G12, M142-H08, X63-G06, X101-A01, X81-B01, X67-D03, X67-G04, X115-B07, X115-D05, X115-E09, X115-H06, X115-A03, X115-D01, X115-F02, X124-G01, X115-G04, M29-D09, M145-D11, M06-D09 and M35-G04.

In a preferred embodiment, the protein is an antibody (e.g., a human antibody) having one or more (e.g., 1, 2, or 3) heavy chain CDRs selected from the corresponding CDRs of the group of heavy chains consisting of M162-A04, M199-A08, M160-G12, M142-H08, X63-G06, X101-A01, X81-B01, X67-D03, X67-G04, X115-B07, X115-D05, X115-E09, X115-H06, X115-A03, X115-D01, X115-F02, X124-G01, X115-G04, M29-D09, M145-D11, M06-D09 and M35-G04 and one or more (e.g., 1, 2, or 3) light chain CDRs selected from the corresponding CDRs of the group of light chains consisting of M162-A04, M199-A08, M160-G12, M142-H08, X63-G06, X101-A01, X81-B01, X67-D03, X67-G04, X115-B07, X115-D05, X115-E09, X115-H06, X115-A03, X115-D01, X115-F02, X124-G01, X115-G04, M29-D09, M145-D11, M06-D09 and M35-G04 (respectively).

In a preferred embodiment, the protein is an antibody (e.g., a human antibody) having the light and heavy chains of X81-B01.

In a preferred embodiment, the protein is an antibody (e.g., a human antibody) having the heavy chain of X81-B01.

In a preferred embodiment, the protein is an antibody (e.g., a human antibody) having the light chain of X81-B01.

In a preferred embodiment, the protein is an antibody (e.g., a human antibody) having light and heavy antibody variable regions of an antibody selected from X81-B01.

In a preferred embodiment, the protein is an antibody (e.g., a human antibody) having a heavy chain antibody variable region of X81-B01.

In a preferred embodiment, the protein is an antibody (e.g., a human antibody) having a light chain antibody variable region of X81-B01.

In a preferred embodiment, the protein is an antibody (e.g., a human antibody) having one or more (e.g., 1, 2, or 3) heavy chain CDRs from the corresponding CDRs of the heavy chain of X81-B01.

In a preferred embodiment, the protein is an antibody (e.g., a human antibody) having one or more (e.g., 1, 2, or 3) light chain CDRs from the corresponding CDRs of the light chain of X81-B01.

In a preferred embodiment, the protein is an antibody (e.g., a human antibody) having one or more (e.g., 1, 2, or 3) heavy chain CDRs from the heavy chain of X81-B01 and one or more (e.g., 1, 2, or 3) light chain CDRs from the corresponding CDRs of the light chain of X81-B01.

In a preferred embodiment, the protein is an antibody (e.g., a human antibody) having the light and heavy chains of X67-D03.

In a preferred embodiment, the protein is an antibody (e.g., a human antibody) having the heavy chain of X67-D03.

In a preferred embodiment, the protein is an antibody (e.g., a human antibody) having the light chain of X67-D03.

In a preferred embodiment, the protein is an antibody (e.g., a human antibody) having light and heavy antibody variable regions of an antibody selected from X67-D03.

In a preferred embodiment, the protein is an antibody (e.g., a human antibody) having a heavy chain antibody variable region of X67-D03.

In a preferred embodiment, the protein is an antibody (e.g., a human antibody) having a light chain antibody variable region of X67-D03.

In a preferred embodiment, the protein is an antibody (e.g., a human antibody) having one or more (e.g., 1, 2, or 3) heavy chain CDRs from the corresponding CDRs of the heavy chain of X67-D03.

In a preferred embodiment, the protein is an antibody (e.g., a human antibody) having one or more (e.g., 1, 2, or 3) light chain CDRs from the corresponding CDRs of the light chain of X67-D03.

In a preferred embodiment, the protein is an antibody (e.g., a human antibody) having one or more (e.g., 1, 2, or 3) heavy chain CDRs from the heavy chain of X67-D03 and one or more (e.g., 1, 2, or 3) light chain CDRs from the corresponding CDRs of the light chain of X67-D03.

In a preferred embodiment, the protein is an antibody (e.g., a human antibody) having the light and heavy chains of X115-F02 or X124-G01.

In a preferred embodiment, the protein is an antibody (e.g., a human antibody) having the heavy chain of X115-F02 or X124-G01.

In a preferred embodiment, the protein is an antibody (e.g., a human antibody) having the light chain of X115-F02 or X124-G01.

In a preferred embodiment, the protein is an antibody (e.g., a human antibody) having light and heavy antibody variable regions of an antibody selected from X115-F02 or X124-G01.

In a preferred embodiment, the protein is an antibody (e.g., a human antibody) having a heavy chain antibody variable region of X115-F02 or X124-G01.

In a preferred embodiment, the protein is an antibody (e.g., a human antibody) having a light chain antibody variable region of X115-F02 or X124-G01.

In a preferred embodiment, the protein is an antibody (e.g., a human antibody) having one or more (e.g., 1, 2, or 3) heavy chain CDRs from the corresponding CDRs of the heavy chain of X115-F02 or X124-G01.

In a preferred embodiment, the protein is an antibody (e.g., a human antibody) having one or more (e.g., 1, 2, or 3) light chain CDRs from the corresponding CDRs of the light chain of X115-F02 or X124-G01.

In a preferred embodiment, the protein is an antibody (e.g., a human antibody) having one or more (e.g., 1, 2, or 3) heavy chain CDRs from the heavy chain of X115-F02 or X124-G01 and one or more (e.g., 1, 2, or 3) light chain CDRs from the corresponding CDRs of the light chain of X115-F02 or X124-G01.

In one embodiment, the HC and LC variable domain sequences are components of the same polypeptide chain.

In some embodiments, the plasma kallikrein binding protein has a serum residence time of 1 week, 2 weeks, 3 weeks, 4 weeks, 5 weeks or more, in vivo, e.g., in humans. In one embodiment, the plasma kallikrein binding protein is an IgG, e.g., an IgG1, IgG2, IgG3 or IgG4, that has a serum residence time of 1 week, 2 weeks, 3 weeks, 4 weeks, 5 weeks or more in vivo, e.g., in humans.

In some embodiments, the plasma kallikrein binding protein is physically associated with a moiety that improves serum residence time, e.g., a moiety described herein.

In another embodiment, the HC and LC variable domain sequences are components of different polypeptide chains. For example, the protein is an IgG, e.g., IgG1, IgG2, IgG3, or IgG4. The protein can be a soluble Fab (sFab).

In other implementations the protein includes a Fab2', scFv, minibody, scFv::Fc fusion, Fab::HSA fusion, HSA::Fab fusion, Fab::HSA::Fab fusion, or other molecule that comprises the antigen combining site of one of the binding proteins herein. The VH and VL regions of these Fabs can be provided as IgG, Fab, Fab2, Fab2', scFv, PEGylated Fab, PEGylated scFv, PEGylated Fab2, VH::CH1::HSA+LC, HSA::VH::CH1+LC, LC::HSA+VH::CH1, HSA::LC+VH::CH1, or other appropriate construction.

In one embodiment, the protein is a human or humanized antibody or is non-immunogenic in a human. For example, the protein includes one or more human antibody framework regions, e.g., all human framework regions.

In one embodiment, the protein includes a human Fc domain, or an Fc domain that is at least 95, 96, 97, 98, or 99% identical to a human Fc domain.

In one embodiment, the protein is a primate or primatized antibody or is non-immunogenic in a human. For example, the protein includes one or more primate antibody framework regions, e.g., all primate framework regions.

In one embodiment, the protein includes a primate Fc domain, or an Fc domain that is at least 95, 96, 97, 98, or 99% identical to a primate Fc domain. "Primate" includes humans (*Homo sapiens*), chimpanzees (*Pan troglodytes* and *Pan paniscus* (bonobos)), gorillas (*Gorilla gorilla*), gibons, monkeys, lemurs, aye-ayes (*Daubentonia madagascariensis*), and tarsiers.

In one embodiment, the protein includes human framework regions, or framework regions that are at least 95, 96, 97, 98, or 99% identical to human framework regions.

In certain embodiments, the protein includes no sequences from mice or rabbits (e.g., is not a murine or rabbit antibody).

In certain embodiments, the protein is capable of binding to a cell or tissue, e.g., that expresses plasma kallikrein.

In one embodiment, protein is physically associated with a nanoparticle, and can be used to guide a nanoparticle to a cell or tissue expressing plasma kallikrein.

In some aspects, the disclosure features a method of promoting wound healing in a subject, the method comprising:

administering an isolated protein (e.g., antibody, e.g., human antibody) that binds plasma kallikrein (e.g., human plasma kallikrein and/or mouse plasma kallikrein) and, e.g., does not bind prekallikrein (e.g., human prekallikrein and/or mouse prekallikrein) to the subject.

In some embodiments, the protein binds the same epitope or competes for binding with a kallikrein binding protein described herein. In some embodiments, the protein binds the same epitope or competes for binding with a protein (e.g., epi-Kal2) and/or a small molecule (e.g., AEBSF) described herein.

In some embodiments, the plasma kallikrein binding protein is administered in combination with another treatment for wound healing.

In some embodiments, the protein described herein is selected from the group consisting of M162-A04, M199-A08, M160-G12, M142-H08, X63-G06, X101-A01, X81-B01, X67-D03, X67-G04, X115-B07, X115-D05, X115-

E09, X115-H06, X115-A03, X115-D01, X115-F02, X124-G01, X115-G04, M29-D09, M145-D11, M06-D09 and M35-G04.

In some embodiments, the plasma kallikrein binding protein competes with or binds the same epitope as X81-B01.

In some embodiments, the plasma kallikrein binding protein competes with or binds the same epitope as X67-D03.

In some embodiments, the plasma kallikrein binding protein competes with or binds the same epitope as M162-A04.

In some embodiments, the plasma kallikrein binding protein competes with or binds the same epitope as X115-F02 or X124-G01.

In some embodiments, the plasma kallikrein binding protein does not bind prekallikrein (e.g., human prekallikrein), but binds to the active form of plasma kallikrein (e.g., human plasma kallikrein).

In certain embodiments, the protein binds at or near the active site of the catalytic domain of plasma kallikrein, or a fragment thereof, or binds an epitope that overlaps with the active site of plasma kallikrein.

In some embodiments, the protein binds to one or more amino acids that form the catalytic triad of plasma kallikrein: His434, Asp483, and/or Ser578 (numbering based on the human sequence). In other embodiments, the protein binds to one or more amino acids that form a region for substrate recognition: Arg551, Gln553, Tyr555, Thr558, and/or Arg560 (numbering based on the human sequence). In some embodiments, the plasma kallikrein binding protein binds one or more amino acids of: S478, N481, S525, and K526 (numbering based on the human kallikrein sequence).

In some embodiments, the protein binds to one or more amino acids of Ser479, Tyr563, and/or Asp585 (numbering based on the human sequence).

In some embodiments, the plasma kallikrein binding protein decreases Factor XIIa and/or bradykinin production by greater than about 5%, about 10%, about 15%, about 20%, about 25%, about 30%, about 35%, about 40%, about 45%, about 50%, about 55%, about 60%, about 65%, about 70%, about 75%, about 80%, about 85%, about 90%, or about 95% as compared to a standard, e.g., the Factor XIIa and/or bradykinin production under the same conditions but in the absence of the protein.

In some embodiments, the plasma kallikrein binding protein has an apparent inhibition constant ($K_{i,app}$) of less than 1000, 500, 100, 10, 5, 1, 0.5 or 0.2 nM.

In one embodiment, the HC and LC variable domain sequences are components of the same polypeptide chain.

In some embodiments, the plasma kallikrein binding protein has a serum residence time of 1 week, 2 weeks, 3 weeks, 4 weeks, 5 weeks or more, in vivo, e.g., in humans. In one embodiment, the plasma kallikrein binding protein is an IgG, e.g., an IgG1, IgG2, IgG3 or IgG4, that has a serum residence time of 1 week, 2 weeks, 3 weeks, 4 weeks, 5 weeks or more in vivo, e.g., in humans.

In some embodiments, the plasma kallikrein binding protein is physically associated with a moiety that improves serum residence time, e.g., a moiety described herein.

In another embodiment, the HC and LC variable domain sequences are components of different polypeptide chains. For example, the plasma kallikrein binding protein is an IgG, e.g., IgG1, IgG2, IgG3, or IgG4. The plasma kallikrein binding protein can be a soluble Fab (sFab).

In other implementations the plasma kallikrein binding protein includes a Fab2', scFv, minibody, scFv::Fc fusion, Fab::HSA fusion, HSA::Fab fusion, Fab::HSA::Fab fusion, or other molecule that comprises the antigen combining site of one of the binding proteins herein. The VH and VL regions of these Fabs can be provided as IgG, Fab, Fab2, Fab2', scFv, PEGylated Fab, PEGylated scFv, PEGylated Fab2, VH::CH1::HSA+LC, HSA::VH::CH1+LC, LC::HSA+VH::CH1, HSA::LC+VH::CH1, or other appropriate construction.

In one embodiment, the plasma kallikrein binding protein is a human or humanized antibody or is non-immunogenic in a human. For example, the protein includes one or more human antibody framework regions, e.g., all human framework regions.

In one embodiment, the plasma kallikrein binding protein includes a human Fc domain, or an Fc domain that is at least 95, 96, 97, 98, or 99% identical to a human Fc domain.

In one embodiment, the plasma kallikrein binding protein is a primate or primatized antibody or is non-immunogenic in a human. For example, the protein includes one or more primate antibody framework regions, e.g., all primate framework regions.

In one embodiment, the plasma kallikrein binding protein includes a primate Fc domain, or an Fc domain that is at least 95, 96, 97, 98, or 99% identical to a primate Fc domain. "Primate" includes humans (*Homo sapiens*), chimpanzees (*Pan troglodytes* and *Pan paniscus* (bonobos)), gorillas (*Gorilla gorilla*), gibons, monkeys, lemurs, aye-ayes (*Daubentonia madagascariensis*), and tarsiers.

In one embodiment, the plasma kallikrein binding protein includes human framework regions, or framework regions that are at least 95, 96, 97, 98, or 99% identical to human framework regions.

In certain embodiments, the plasma kallikrein binding protein includes no sequences from mice or rabbits (e.g., is not a murine or rabbit antibody).

In certain embodiments, the protein is capable of binding to a cell or tissue, e.g., that expresses plasma kallikrein.

In one embodiment, the plasma kallikrein binding protein is physically associated with a nanoparticle, and can be used to guide a nanoparticle to a cell or tissue expressing plasma kallikrein.

In some aspects, the disclosure features a method promoting wound healing in a subject, the method comprising:

administering an isolated protein (e.g., antibody, e.g., human antibody) comprising a heavy chain immunoglobulin variable domain sequence and a light chain immunoglobulin variable domain sequence to the subject, wherein:

the heavy chain immunoglobulin variable domain sequence comprises one, two, or three (e.g., three) CDR regions from the heavy chain variable domain of a protein described herein, and/or

the light chain immunoglobulin variable domain sequence comprises one, two, or three (e.g., three) CDR regions from the light chain variable domain of a protein described herein,

wherein the protein binds to plasma kallikrein.

In some embodiments, the protein is administered in combination with another treatment for wound healing.

In some embodiments, the heavy chain immunoglobulin variable domain sequence comprises one, two, or three (e.g., three) CDR regions from the heavy chain variable domain of M162-A04, M199-A08, M160-G12, M142-H08, X63-G06, X101-A01, X81-B01, X67-D03, X67-G04, X115-B07, X115-D05, X115-E09, X115-H06, X115-A03, X115-D01, X115-F02, X124-G01, X115-G04, M29-D09, M145-D11, M06-D09 and M35-G04, and/or

the light chain immunoglobulin variable domain sequence comprises one, two, or three (e.g., three) CDR regions from the light chain variable domain of M162-A04, M199-A08, M160-G12, M142-H08, X63-G06, X101-A01, X81-B01, X67-D03, X67-G04, X115-B07, X115-D05, X115-E09,

X115-H06, X115-A03, X115-D01, X115-F02, X124-G01, X115-G04, M29-D09, M145-D11, M06-D09 and M35-G04 (respectively).

In some embodiments, the protein inhibits plasma kallikrein.

In some embodiments, the one, two, or three (e.g., three) CDR regions from the heavy chain variable domain are from X81-B01 and/or the one, two, or three (e.g., three) CDR regions from the light chain variable domain are from X81-B01.

In some embodiments, the one, two, or three (e.g., three) CDR regions from the heavy chain variable domain are from X67-D03 and/or the one, two, or three (e.g., three) CDR regions from the light chain variable domain are from X67-D03.

In some embodiments, the one, two, or three (e.g., three) CDR regions from the heavy chain variable domain are from M162-A04 and/or the one, two, or three (e.g., three) CDR regions from the light chain variable domain are from M162-A04.

In some embodiments, the one, two, or three (e.g., three) CDR regions from the heavy chain variable domain are from M199-A08 and/or the one, two, or three (e.g., three) CDR regions from the light chain variable domain are from M199-A08.

In some embodiments, the one, two, or three (e.g., three) CDR regions from the heavy chain variable domain are from X115-F02 or X124-G01 and/or the one, two, or three (e.g., three) CDR regions from the light chain variable domain are from X115-F02 or X124-G01.

In some embodiments, the heavy chain immunoglobulin variable domain sequence comprises the heavy chain variable domain of a protein described herein, and/or the light chain immunoglobulin variable domain sequence comprises the light chain variable domain of a protein described herein.

In some embodiments, the heavy chain immunoglobulin variable domain sequence comprises the heavy chain variable domain of M162-A04, M199-A08, M160-G12, M142-H08, X63-G06, X101-A01, X81-B01, X67-D03, X67-G04, X115-B07, X115-D05, X115-E09, X115-H06, X115-A03, X115-D01, X115-F02, X124-G01, X115-G04, M29-D09, M145-D11, M06-D09 and M35-G04, and/or the light chain immunoglobulin variable domain sequence comprises the light chain variable domain of M162-A04, M199-A08, M160-G12, M142-H08, X63-G06, X101-A01, X81-B01, X67-D03, X67-G04, X115-B07, X115-D05, X115-E09, X115-H06, X115-A03, X115-D01, X115-F02, X124-G01, X115-G04, M29-D09, M145-D11, M06-D09 and M35-G04 (respectively).

In some embodiments, the heavy chain immunoglobulin variable domain sequence comprises the heavy chain variable domain of X81-B01, and/or the light chain immunoglobulin variable domain sequence comprises the light chain variable domain of X81-B01.

In some embodiments, the heavy chain immunoglobulin variable domain sequence comprises the heavy chain variable domain of X67-D03, and/or the light chain immunoglobulin variable domain sequence comprises the light chain variable domain of X67-D03.

In some embodiments, the protein comprises the heavy chain of a protein described herein, and/or the light chain of a protein described herein.

In some embodiments, the protein comprises the heavy chain of M162-A04, M199-A08, M160-G12, M142-H08, X63-G06, X101-A01, X81-B01, X67-D03, X67-G04, X115-B07, X115-D05, X115-E09, X115-H06, X115-A03, X115-D01, X115-F02, X124-G01, X115-G04, M29-D09, M145-D11, M06-D09 and M35-G04, and/or the light chain of M162-A04, M199-A08, M160-G12, M142-H08, X63-G06, X101-A01, X81-B01, X67-D03, X67-G04, X115-B07, X115-D05, X115-E09, X115-H06, X115-A03, X115-D01, X115-F02, X124-G01, X115-G04, M29-D09, M145-D11, M06-D09 and M35-G04 (respectively).

In some embodiments, the protein comprises the heavy chain of X81-B01, and/or the light chain of X81-B01.

In some embodiments, the protein comprises the heavy chain of X67-D03, and/or the light chain of X67-D03.

In some embodiments, the protein comprises the heavy chain of M162-A04, and/or the light chain of M162-A04.

In some embodiments, the protein comprises the heavy chain of X115-F02 or X124-G01, and/or the light chain of X115-F02 or X124-G01.

In some embodiments, the plasma kallikrein binding protein does not bind prekallikrein (e.g., human prekallikrein and/or murine prekallikrein), but binds to the active form of plasma kallikrein (e.g., human plasma kallikrein and/or murine plasma kallikrein).

In some embodiments, the plasma kallikrein binding protein decreases Factor XIIa and/or bradykinin production by greater than about 5%, about 10%, about 15%, about 20%, about 25%, about 30%, about 35%, about 40%, about 45%, about 50%, about 55%, about 60%, about 65%, about 70%, about 75%, about 80%, about 85%, about 90%, or about 95% as compared to a standard, e.g., the Factor XIIa and/or bradykinin production under the same conditions but in the absence of the protein.

In some embodiments, the protein includes one or more of the following characteristics: (a) a human CDR or human framework region; (b) the HC immunoglobulin variable domain sequence comprises one or more (e.g., 1, 2, or 3) CDRs that are at least 85, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, or 100% identical to a CDR of a HC variable domain described herein; (c) the LC immunoglobulin variable domain sequence comprises one or more (e.g., 1, 2, or 3) CDRs that are at least 85, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, or 100% identical to a CDR of a LC variable domain described herein; (d) the LC immunoglobulin variable domain sequence is at least 85, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, or 100% identical to a LC variable domain described herein (e.g., overall or in framework regions or CDRs); (e) the HC immunoglobulin variable domain sequence is at least 85, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, or 100% identical to a HC variable domain described herein (e.g., overall or in framework regions or CDRs); (f) the protein binds an epitope bound by a protein described herein, or competes for binding with a protein described herein; and (g) a primate CDR or primate framework region.

In some embodiments, the protein has an apparent inhibition constant ($K_{i,app}$) of less than 1000, 500, 100, 5, 1, 0.5 or 0.2 nM.

In some embodiments, the antibody does not bind prekallikrein (e.g., human prekallikrein and/or murine prekallikrein), but binds to the active form of plasma kallikrein (e.g., human plasma kallikrein and/or murine plasma kallikrein).

In a preferred embodiment, the protein is an antibody (e.g., a human antibody) having the light and heavy chains of antibodies selected from the group consisting of M162-A04, M199-A08, M160-G12, M142-H08, X63-G06, X101-A01, X81-B01, X67-D03, X67-G04, X115-B07, X115-D05, X115-E09, X115-H06, X115-A03, X115-D01, X115-F02, X-124-G01, X115-G04, M29-D09, M145-D11, M06-D09 and M35-G04.

In a preferred embodiment, the protein is an antibody (e.g., a human antibody) having the heavy chain of an antibody selected from the group consisting of: M162-A04, M199-A08, M160-G12, M142-H08, X63-G06, X101-A01, X81-B01, X67-D03, X67-G04, X115-B07, X115-D05, X115-E09, X115-H06, X115-A03, X115-D01, X115-F02, X124-G01, X115-G04, M29-D09, M145-D11, M06-D09 and M35-G04.

In a preferred embodiment, the protein is an antibody (e.g., a human antibody) having the light chain of an antibody selected from the group consisting of: M162-A04, M199-A08, M160-G12, M142-H08, X63-G06, X101-A01, X81-B01, X67-D03, X67-G04, X115-B07, X115-D05, X115-E09, X115-H06, X115-A03, X115-D01, X115-F02, X124-G01, X115-G04, M29-D09, M145-D11, M06-D09 and M35-G04.

In a preferred embodiment, the protein is an antibody (e.g., a human antibody) having light and heavy antibody variable regions of an antibody selected from the group consisting of M162-A04, M199-A08, M160-G12, M142-H08, X63-G06, X101-A01, X81-B01, X67-D03, X67-G04, X115-B07, X115-D05, X115-E09, X115-H06, X115-A03, X115-D01, X115-F02, X124-G01, X115-G04, M29-D09, M145-D11, M06-D09 and M35-G04.

In a preferred embodiment, the protein is an antibody (e.g., a human antibody) having a heavy chain antibody variable region of an antibody selected from the group consisting of: M162-A04, M199-A08, M160-G12, M142-H08, X63-G06, X101-A01, X81-B01, X67-D03, X67-G04, X115-B07, X115-D05, X115-E09, X115-H06, X115-A03, X115-D01, X115-F02, X124-G01, X115-G04, M29-D09, M145-D11, M06-D09 and M35-G04.

In a preferred embodiment, the protein is an antibody (e.g., a human antibody) having a light chain antibody variable region of an antibody selected from the group consisting of: M162-A04, M199-A08, M160-G12, M142-H08, X63-G06, X101-A01, X81-B01, X67-D03, X67-G04, X115-B07, X115-D05, X115-E09, X115-H06, X115-A03, X115-D01, X115-F02, X124-G01, X115-G04, M29-D09, M145-D11, M06-D09 and M35-G04.

In a preferred embodiment, the protein is an antibody (e.g., a human antibody) having one or more (e.g., 1, 2, or 3) heavy chain CDRs selected from the corresponding CDRs of the group of heavy chains consisting of M162-A04, M199-A08, M160-G12, M142-H08, X63-G06, X101-A01, X81-B01, X67-D03, X67-G04, X115-B07, X115-D05, X115-E09, X115-H06, X115-A03, X115-D01, X115-F02, X124-G01, X115-G04, M29-D09, M145-D11, M06-D09 and M35-G04.

In a preferred embodiment, the protein is an antibody (e.g., a human antibody) having one or more (e.g., 1, 2, or 3) light chain CDRs selected from the corresponding CDRs of the group of light chains consisting of M162-A04, M199-A08, M160-G12, M142-H08, X63-G06, X101-A01, X81-B01, X67-D03, X67-G04, X115-B07, X115-D05, X115-E09, X115-H06, X115-A03, X115-D01, X115-F02, X124-G01, X115-G04, M29-D09, M145-D11, M06-D09 and M35-G04.

In a preferred embodiment, the protein is an antibody (e.g., a human antibody) having one or more (e.g., 1, 2, or 3) heavy chain CDRs selected from the corresponding CDRs of the group of heavy chains consisting of M162-A04, M199-A08, M160-G12, M142-H08, X63-G06, X101-A01, X81-B01, X67-D03, X67-G04, X115-B07, X115-D05, X115-E09, X115-H06, X115-A03, X115-D01, X115-F02, X124-G01, X115-G04, M29-D09, M145-D11, M06-D09 and M35-G04 and one or more (e.g., 1, 2, or 3) light chain CDRs selected from the corresponding CDRs of the group of light chains consisting of M162-A04, M199-A08, M160-G12, M142-H08, X63-G06, X101-A01, X81-B01, X67-D03, X67-G04, X115-B07, X115-D05, X115-E09, X115-H06, X115-A03, X115-D01, X115-F02, X124-G01, X115-G04, M29-D09, M145-D11, M06-D09 and M35-G04 (respectively).

In a preferred embodiment, the protein is an antibody (e.g., a human antibody) having the light and heavy chains of X81-B01.

In a preferred embodiment, the protein is an antibody (e.g., a human antibody) having the heavy chain of X81-B01.

In a preferred embodiment, the protein is an antibody (e.g., a human antibody) having the light chain of X81-B01.

In a preferred embodiment, the protein is an antibody (e.g., a human antibody) having light and heavy antibody variable regions of an antibody selected from X81-B01.

In a preferred embodiment, the protein is an antibody (e.g., a human antibody) having a heavy chain antibody variable region of X81-B01.

In a preferred embodiment, the protein is an antibody (e.g., a human antibody) having a light chain antibody variable region of X81-B01.

In a preferred embodiment, the protein is an antibody (e.g., a human antibody) having one or more (e.g., 1, 2, or 3) heavy chain CDRs from the corresponding CDRs of the heavy chain of X81-B01.

In a preferred embodiment, the protein is an antibody (e.g., a human antibody) having one or more (e.g., 1, 2, or 3) light chain CDRs from the corresponding CDRs of the light chain of X81-B01.

In a preferred embodiment, the protein is an antibody (e.g., a human antibody) having one or more (e.g., 1, 2, or 3) heavy chain CDRs from the heavy chain of X81-B01 and one or more (e.g., 1, 2, or 3) light chain CDRs from the corresponding CDRs of the light chain of X81-B01.

In a preferred embodiment, the protein is an antibody (e.g., a human antibody) having the light and heavy chains of X67-D03.

In a preferred embodiment, the protein is an antibody (e.g., a human antibody) having the heavy chain of X67-D03.

In a preferred embodiment, the protein is an antibody (e.g., a human antibody) having the light chain of X67-D03.

In a preferred embodiment, the protein is an antibody (e.g., a human antibody) having light and heavy antibody variable regions of an antibody selected from X67-D03.

In a preferred embodiment, the protein is an antibody (e.g., a human antibody) having a heavy chain antibody variable region of X67-D03.

In a preferred embodiment, the protein is an antibody (e.g., a human antibody) having a light chain antibody variable region of X67-D03.

In a preferred embodiment, the protein is an antibody (e.g., a human antibody) having one or more (e.g., 1, 2, or 3) heavy chain CDRs from the corresponding CDRs of the heavy chain of X67-D03.

In a preferred embodiment, the protein is an antibody (e.g., a human antibody) having one or more (e.g., 1, 2, or 3) light chain CDRs from the corresponding CDRs of the light chain of X67-D03.

In a preferred embodiment, the protein is an antibody (e.g., a human antibody) having one or more (e.g., 1, 2, or 3) heavy chain CDRs from the heavy chain of X67-D03 and one or more (e.g., 1, 2, or 3) light chain CDRs from the corresponding CDRs of the light chain of X67-D03.

In a preferred embodiment, the protein is an antibody (e.g., a human antibody) having the light and heavy chains of X115-F02 or X124-G01.

In a preferred embodiment, the protein is an antibody (e.g., a human antibody) having the heavy chain of X115-F02 or X124-G01.

In a preferred embodiment, the protein is an antibody (e.g., a human antibody) having the light chain of X115-F02 or X124-G01.

In a preferred embodiment, the protein is an antibody (e.g., a human antibody) having light and heavy antibody variable regions of an antibody selected from X115-F02 or X124-G01.

In a preferred embodiment, the protein is an antibody (e.g., a human antibody) having a heavy chain antibody variable region of X115-F02 or X124-G01.

In a preferred embodiment, the protein is an antibody (e.g., a human antibody) having a light chain antibody variable region of X115-F02 or X124-G01.

In a preferred embodiment, the protein is an antibody (e.g., a human antibody) having one or more (e.g., 1, 2, or 3) heavy chain CDRs from the corresponding CDRs of the heavy chain of X115-F02 or X124-G01.

In a preferred embodiment, the protein is an antibody (e.g., a human antibody) having one or more (e.g., 1, 2, or 3) light chain CDRs from the corresponding CDRs of the light chain of X115-F02 or X124-G01.

In a preferred embodiment, the protein is an antibody (e.g., a human antibody) having one or more (e.g., 1, 2, or 3) heavy chain CDRs from the heavy chain of X115-F02 or X124-G01 and one or more (e.g., 1, 2, or 3) light chain CDRs from the corresponding CDRs of the light chain of X115-F02 or X124-G01.

In one embodiment, the HC and LC variable domain sequences are components of the same polypeptide chain.

In some embodiments, the plasma kallikrein binding protein has a serum residence time of 1 week, 2 weeks, 3 weeks, 4 weeks, 5 weeks or more, in vivo, e.g., in humans. In one embodiment, the plasma kallikrein binding protein is an IgG, e.g., an IgG1, IgG2, IgG3 or IgG4, that has a serum residence time of 1 week, 2 weeks, 3 weeks, 4 weeks, 5 weeks or more in vivo, e.g., in humans.

In some embodiments, the plasma kallikrein binding protein is physically associated with a moiety that improves serum residence time, e.g., a moiety described herein.

In another embodiment, the HC and LC variable domain sequences are components of different polypeptide chains. For example, the protein is an IgG, e.g., IgG1, IgG2, IgG3, or IgG4. The protein can be a soluble Fab (sFab).

In other implementations the protein includes a Fab2', scFv, minibody, scFv::Fc fusion, Fab::HSA fusion, HSA::Fab fusion, Fab::HSA::Fab fusion, or other molecule that comprises the antigen combining site of one of the binding proteins herein. The VH and VL regions of these Fabs can be provided as IgG, Fab, Fab2, Fab2', scFv, PEGylated Fab, PEGylated scFv, PEGylated Fab2, VH::CH1::HSA+LC, HSA::VH::CH1+LC, LC::HSA+VH::CH1, HSA::LC+VH::CH1, or other appropriate construction.

In one embodiment, the protein is a human or humanized antibody or is non-immunogenic in a human. For example, the protein includes one or more human antibody framework regions, e.g., all human framework regions.

In one embodiment, the protein includes a human Fc domain, or an Fc domain that is at least 95, 96, 97, 98, or 99% identical to a human Fc domain.

In one embodiment, the protein is a primate or primatized antibody or is non-immunogenic in a human. For example, the protein includes one or more primate antibody framework regions, e.g., all primate framework regions.

In one embodiment, the protein includes a primate Fc domain, or an Fc domain that is at least 95, 96, 97, 98, or 99% identical to a primate Fc domain. "Primate" includes humans (*Homo sapiens*), chimpanzees (*Pan troglodytes* and *Pan paniscus* (bonobos)), gorillas (*Gorilla gorilla*), gibons, monkeys, lemurs, aye-ayes (*Daubentonia madagascariensis*), and tarsiers.

In one embodiment, the protein includes human framework regions, or framework regions that are at least 95, 96, 97, 98, or 99% identical to human framework regions.

In certain embodiments, the protein includes no sequences from mice or rabbits (e.g., is not a murine or rabbit antibody).

In certain embodiments, the protein is capable of binding to a cell or tissue, e.g., that expresses plasma kallikrein.

In one embodiment, protein is physically associated with a nanoparticle, and can be used to guide a nanoparticle to a cell or tissue expressing plasma kallikrein.

In some aspects, the disclosure features a method of treating or preventing rheumatoid arthritis in a subject, the method comprising:

administering an isolated protein (e.g., antibody, e.g., human antibody) comprising a heavy chain immunoglobulin variable domain sequence and a light chain immunoglobulin variable domain sequence to the subject, wherein:

the heavy chain immunoglobulin variable domain sequence comprises one, two, or three (e.g., three) CDR regions from the heavy chain variable domain of a protein described herein, and/or

the light chain immunoglobulin variable domain sequence comprises one, two, or three (e.g., three) CDR regions from the light chain variable domain of a protein described herein,

wherein the protein binds to plasma kallikrein.

In some embodiments, the plasma kallikrein binding protein does not bind prekallikrein (e.g., human prekallikrein and/or murine prekallikrein), but binds to the active form of plasma kallikrein (e.g., human plasma kallikrein and/or murine plasma kallikrein).

In some embodiments, the protein is administered in combination with another treatment for rheumatoid arthritis.

In some embodiments, the protein inhibits plasma kallikrein (e.g., human plasma kallikrein and/or murine plasma kallikrein).

In some embodiments, the heavy chain immunoglobulin variable domain sequence comprises the heavy chain variable domain of a protein described herein, and/or the light chain immunoglobulin variable domain sequence comprises the light chain variable domain of a protein described herein.

In some embodiments, the protein comprises the heavy chain of a protein described herein, and/or the light chain of a protein described herein.

In some aspects, the disclosure features a method of treating or preventing gout in a subject, the method comprising:

administering an isolated protein (e.g., antibody, e.g., human antibody) comprising a heavy chain immunoglobulin variable domain sequence and a light chain immunoglobulin variable domain sequence to the subject, wherein:

the heavy chain immunoglobulin variable domain sequence comprises one, two, or three (e.g., three) CDR regions from the heavy chain variable domain of a protein described herein, and/or

the light chain immunoglobulin variable domain sequence comprises one, two, or three (e.g., three) CDR regions from the light chain variable domain of a protein described herein,

wherein the protein binds to plasma kallikrein.

In some embodiments, the plasma kallikrein binding protein does not bind prekallikrein (e.g., human prekallikrein and/or murine prekallikrein), but binds to the active form of plasma kallikrein (e.g., human plasma kallikrein and/or murine plasma kallikrein).

In some embodiments, the protein is administered in combination with another treatment for gout.

In some embodiments, the protein inhibits plasma kallikrein (e.g., human plasma kallikrein and/or murine plasma kallikrein).

In some embodiments, the heavy chain immunoglobulin variable domain sequence comprises the heavy chain variable domain of a protein described herein, and/or the light chain immunoglobulin variable domain sequence comprises the light chain variable domain of a protein described herein.

In some embodiments, the protein comprises the heavy chain of a protein described herein, and/or the light chain of a protein described herein.

In some aspects, the disclosure features a method of treating or preventing intestinal bowel disease in a subject, the method comprising:

administering an isolated protein (e.g., antibody, e.g., human antibody) comprising a heavy chain immunoglobulin variable domain sequence and a light chain immunoglobulin variable domain sequence to the subject, wherein:

the heavy chain immunoglobulin variable domain sequence comprises one, two, or three (e.g., three) CDR regions from the heavy chain variable domain of a protein described herein, and/or

the light chain immunoglobulin variable domain sequence comprises one, two, or three (e.g., three) CDR regions from the light chain variable domain of a protein described herein,

wherein the protein binds to plasma kallikrein.

In some embodiments, the plasma kallikrein binding protein does not bind prekallikrein (e.g., human prekallikrein and/or murine prekallikrein), but binds to the active form of plasma kallikrein (e.g., human plasma kallikrein and/or murine plasma kallikrein).

In some embodiments, the protein is administered in combination with another treatment for intestinal bowel disease.

In some embodiments, the protein inhibits plasma kallikrein (e.g., human plasma kallikrein and/or murine plasma kallikrein).

In some embodiments, the heavy chain immunoglobulin variable domain sequence comprises the heavy chain variable domain of a protein described herein, and/or the light chain immunoglobulin variable domain sequence comprises the light chain variable domain of a protein described herein.

In some embodiments, the protein comprises the heavy chain of a protein described herein, and/or the light chain of a protein described herein.

In some aspects, the disclosure features a method of treating or preventing oral mucositis in a subject, the method comprising:

administering an isolated protein (e.g., antibody, e.g., human antibody) comprising a heavy chain immunoglobulin variable domain sequence and a light chain immunoglobulin variable domain sequence to the subject, wherein:

the heavy chain immunoglobulin variable domain sequence comprises one, two, or three (e.g., three) CDR regions from the heavy chain variable domain of a protein described herein, and/or

the light chain immunoglobulin variable domain sequence comprises one, two, or three (e.g., three) CDR regions from the light chain variable domain of a protein described herein,

wherein the protein binds to plasma kallikrein.

In some embodiments, the plasma kallikrein binding protein does not bind prekallikrein (e.g., human prekallikrein and/or murine prekallikrein), but binds to the active form of plasma kallikrein (e.g., human plasma kallikrein and/or murine plasma kallikrein).

In some embodiments, the protein is administered in combination with another treatment for oral mucositis.

In some embodiments, the protein inhibits plasma kallikrein (e.g., human plasma kallikrein and/or murine plasma kallikrein).

In some embodiments, the heavy chain immunoglobulin variable domain sequence comprises the heavy chain variable domain of a protein described herein, and/or the light chain immunoglobulin variable domain sequence comprises the light chain variable domain of a protein described herein.

In some embodiments, the protein comprises the heavy chain of a protein described herein, and/or the light chain of a protein described herein.

In some aspects, the disclosure features a method of treating or preventing neuropathic pain in a subject, the method comprising:

administering an isolated protein (e.g., antibody, e.g., human antibody) comprising a heavy chain immunoglobulin variable domain sequence and a light chain immunoglobulin variable domain sequence to the subject, wherein:

the heavy chain immunoglobulin variable domain sequence comprises one, two, or three (e.g., three) CDR regions from the heavy chain variable domain of a protein described herein, and/or

the light chain immunoglobulin variable domain sequence comprises one, two, or three (e.g., three) CDR regions from the light chain variable domain of a protein described herein,

wherein the protein binds to plasma kallikrein.

In some embodiments, the plasma kallikrein binding protein does not bind prekallikrein (e.g., human prekallikrein and/or murine prekallikrein), but binds to the active form of plasma kallikrein (e.g., human plasma kallikrein and/or murine plasma kallikrein).

In some embodiments, the protein is administered in combination with another treatment for neuropathic pain.

In some embodiments, the protein inhibits plasma kallikrein (e.g., human plasma kallikrein and/or murine plasma kallikrein).

In some embodiments, the heavy chain immunoglobulin variable domain sequence comprises the heavy chain variable domain of a protein described herein, and/or the light chain immunoglobulin variable domain sequence comprises the light chain variable domain of a protein described herein.

In some embodiments, the protein comprises the heavy chain of a protein described herein, and/or the light chain of a protein described herein.

In some aspects, the disclosure features a method of treating or preventing inflammatory pain in a subject, the method comprising:

administering an isolated protein (e.g., antibody, e.g., human antibody) comprising a heavy chain immunoglobulin variable domain sequence and a light chain immunoglobulin variable domain sequence to the subject, wherein:

the heavy chain immunoglobulin variable domain sequence comprises one, two, or three (e.g., three) CDR regions from the heavy chain variable domain of a protein described herein, and/or

the light chain immunoglobulin variable domain sequence comprises one, two, or three (e.g., three) CDR regions from the light chain variable domain of a protein described herein,

wherein the protein binds to plasma kallikrein.

In some embodiments, the plasma kallikrein binding protein does not bind prekallikrein (e.g., human prekallikrein and/or murine prekallikrein), but binds to the active form of plasma kallikrein (e.g., human plasma kallikrein and/or murine plasma kallikrein).

In some embodiments, the protein is administered in combination with another treatment for inflammatory pain.

In some embodiments, the protein inhibits plasma kallikrein (e.g., human plasma kallikrein and/or murine plasma kallikrein).

In some embodiments, the heavy chain immunoglobulin variable domain sequence comprises the heavy chain variable domain of a protein described herein, and/or the light chain immunoglobulin variable domain sequence comprises the light chain variable domain of a protein described herein.

In some embodiments, the protein comprises the heavy chain of a protein described herein, and/or the light chain of a protein described herein.

In some aspects, the disclosure features a method of treating or preventing spinal stenosis-degenerative spine disease in a subject, the method comprising:

administering an isolated protein (e.g., antibody, e.g., human antibody) comprising a heavy chain immunoglobulin variable domain sequence and a light chain immunoglobulin variable domain sequence to the subject, wherein:

the heavy chain immunoglobulin variable domain sequence comprises one, two, or three (e.g., three) CDR regions from the heavy chain variable domain of a protein described herein, and/or

the light chain immunoglobulin variable domain sequence comprises one, two, or three (e.g., three) CDR regions from the light chain variable domain of a protein described herein,

wherein the protein binds to plasma kallikrein.

In some embodiments, the plasma kallikrein binding protein does not bind prekallikrein (e.g., human prekallikrein and/or murine prekallikrein), but binds to the active form of plasma kallikrein (e.g., human plasma kallikrein and/or murine plasma kallikrein).

In some embodiments, the protein is administered in combination with another treatment for spinal stenosis-degenerative spine disease.

In some embodiments, the protein inhibits plasma kallikrein (e.g., human plasma kallikrein and/or murine plasma kallikrein).

In some embodiments, the heavy chain immunoglobulin variable domain sequence comprises the heavy chain variable domain of a protein described herein, and/or the light chain immunoglobulin variable domain sequence comprises the light chain variable domain of a protein described herein.

In some embodiments, the protein comprises the heavy chain of a protein described herein, and/or the light chain of a protein described herein.

In some aspects, the disclosure features a method of treating or preventing arterial or venous thrombosis in a subject, the method comprising:

administering an isolated protein (e.g., antibody, e.g., human antibody) comprising a heavy chain immunoglobulin variable domain sequence and a light chain immunoglobulin variable domain sequence to the subject, wherein:

the heavy chain immunoglobulin variable domain sequence comprises one, two, or three (e.g., three) CDR regions from the heavy chain variable domain of a protein described herein, and/or

the light chain immunoglobulin variable domain sequence comprises one, two, or three (e.g., three) CDR regions from the light chain variable domain of a protein described herein,

wherein the protein binds to plasma kallikrein.

In some embodiments, the plasma kallikrein binding protein does not bind prekallikrein (e.g., human prekallikrein and/or murine prekallikrein), but binds to the active form of plasma kallikrein (e.g., human plasma kallikrein and/or murine plasma kallikrein).

In some embodiments, the protein is administered in combination with another treatment for arterial or venous thrombosis.

In some embodiments, the protein inhibits plasma kallikrein (e.g., human plasma kallikrein and/or murine plasma kallikrein).

In some embodiments, the heavy chain immunoglobulin variable domain sequence comprises the heavy chain variable domain of a protein described herein, and/or the light chain immunoglobulin variable domain sequence comprises the light chain variable domain of a protein described herein.

In some embodiments, the protein comprises the heavy chain of a protein described herein, and/or the light chain of a protein described herein.

In some aspects, the disclosure features a method of treating or preventing post operative ileus in a subject, the method comprising:

administering an isolated protein (e.g., antibody, e.g., human antibody) comprising a heavy chain immunoglobulin variable domain sequence and a light chain immunoglobulin variable domain sequence to the subject, wherein:

the heavy chain immunoglobulin variable domain sequence comprises one, two, or three (e.g., three) CDR regions from the heavy chain variable domain of a protein described herein, and/or

the light chain immunoglobulin variable domain sequence comprises one, two, or three (e.g., three) CDR regions from the light chain variable domain of a protein described herein,

wherein the protein binds to plasma kallikrein.

In some embodiments, the plasma kallikrein binding protein does not bind prekallikrein (e.g., human prekallikrein and/or murine prekallikrein), but binds to the active form of plasma kallikrein (e.g., human plasma kallikrein and/or murine plasma kallikrein).

In some embodiments, the protein is administered in combination with another treatment for post operative ileus.

In some embodiments, the protein inhibits plasma kallikrein (e.g., human plasma kallikrein and/or murine plasma kallikrein).

In some embodiments, the heavy chain immunoglobulin variable domain sequence comprises the heavy chain variable domain of a protein described herein, and/or the light chain immunoglobulin variable domain sequence comprises the light chain variable domain of a protein described herein.

In some embodiments, the protein comprises the heavy chain of a protein described herein, and/or the light chain of a protein described herein.

In some aspects, the disclosure features a method of treating or preventing aortic aneurysm in a subject, the method comprising:

administering an isolated protein (e.g., antibody, e.g., human antibody) comprising a heavy chain immunoglobulin variable domain sequence and a light chain immunoglobulin variable domain sequence to the subject, wherein:

the heavy chain immunoglobulin variable domain sequence comprises one, two, or three (e.g., three) CDR regions from the heavy chain variable domain of a protein described herein, and/or

the light chain immunoglobulin variable domain sequence comprises one, two, or three (e.g., three) CDR regions from the light chain variable domain of a protein described herein,

wherein the protein binds to plasma kallikrein.

In some embodiments, the plasma kallikrein binding protein does not bind prekallikrein (e.g., human prekallikrein and/or murine prekallikrein), but binds to the active form of plasma kallikrein (e.g., human plasma kallikrein and/or murine plasma kallikrein).

In some embodiments, the protein is administered in combination with another treatment for aortic aneurysm.

In some embodiments, the protein inhibits plasma kallikrein (e.g., human plasma kallikrein and/or murine plasma kallikrein).

In some embodiments, the heavy chain immunoglobulin variable domain sequence comprises the heavy chain variable domain of a protein described herein, and/or the light chain immunoglobulin variable domain sequence comprises the light chain variable domain of a protein described herein.

In some embodiments, the protein comprises the heavy chain of a protein described herein, and/or the light chain of a protein described herein.

In some aspects, the disclosure features a method of treating or preventing osteoarthritis in a subject, the method comprising:

administering an isolated protein (e.g., antibody, e.g., human antibody) comprising a heavy chain immunoglobulin variable domain sequence and a light chain immunoglobulin variable domain sequence to the subject, wherein:

the heavy chain immunoglobulin variable domain sequence comprises one, two, or three (e.g., three) CDR regions from the heavy chain variable domain of a protein described herein, and/or

the light chain immunoglobulin variable domain sequence comprises one, two, or three (e.g., three) CDR regions from the light chain variable domain of a protein described herein,

wherein the protein binds to plasma kallikrein.

In some embodiments, the plasma kallikrein binding protein does not bind prekallikrein (e.g., human prekallikrein and/or murine prekallikrein), but binds to the active form of plasma kallikrein (e.g., human plasma kallikrein and/or murine plasma kallikrein).

In some embodiments, the protein is administered in combination with another treatment for osteoarthritis.

In some embodiments, the protein inhibits plasma kallikrein (e.g., human plasma kallikrein and/or murine plasma kallikrein).

In some embodiments, the heavy chain immunoglobulin variable domain sequence comprises the heavy chain variable domain of a protein described herein, and/or the light chain immunoglobulin variable domain sequence comprises the light chain variable domain of a protein described herein.

In some embodiments, the protein comprises the heavy chain of a protein described herein, and/or the light chain of a protein described herein.

In some aspects, the disclosure features a method of treating or preventing vasculitis in a subject, the method comprising:

administering an isolated protein (e.g., antibody, e.g., human antibody) comprising a heavy chain immunoglobulin variable domain sequence and a light chain immunoglobulin variable domain sequence to the subject, wherein:

the heavy chain immunoglobulin variable domain sequence comprises one, two, or three (e.g., three) CDR regions from the heavy chain variable domain of a protein described herein, and/or

the light chain immunoglobulin variable domain sequence comprises one, two, or three (e.g., three) CDR regions from the light chain variable domain of a protein described herein,

wherein the protein binds to plasma kallikrein.

In some embodiments, the plasma kallikrein binding protein does not bind prekallikrein (e.g., human prekallikrein and/or murine prekallikrein), but binds to the active form of plasma kallikrein (e.g., human plasma kallikrein and/or murine plasma kallikrein).

In some embodiments, the protein is administered in combination with another treatment for vasculitis.

In some embodiments, the protein inhibits plasma kallikrein (e.g., human plasma kallikrein and/or murine plasma kallikrein).

In some embodiments, the heavy chain immunoglobulin variable domain sequence comprises the heavy chain variable domain of a protein described herein, and/or the light chain immunoglobulin variable domain sequence comprises the light chain variable domain of a protein described herein.

In some embodiments, the protein comprises the heavy chain of a protein described herein, and/or the light chain of a protein described herein.

In some aspects, the disclosure features a method of treating or preventing head trauma or peri-tumor brain edema in a subject, the method comprising:

administering an isolated protein (e.g., antibody, e.g., human antibody) comprising a heavy chain immunoglobulin variable domain sequence and a light chain immunoglobulin variable domain sequence to the subject, wherein:

the heavy chain immunoglobulin variable domain sequence comprises one, two, or three (e.g., three) CDR regions from the heavy chain variable domain of a protein described herein, and/or

the light chain immunoglobulin variable domain sequence comprises one, two, or three (e.g., three) CDR regions from the light chain variable domain of a protein described herein,

wherein the protein binds to plasma kallikrein.

In some embodiments, the plasma kallikrein binding protein does not bind prekallikrein (e.g., human prekallikrein and/or murine prekallikrein), but binds to the active form of plasma kallikrein (e.g., human plasma kallikrein and/or murine plasma kallikrein).

In some embodiments, the protein is administered in combination with another treatment for head trauma or peri-tumor brain edema.

In some embodiments, the protein inhibits plasma kallikrein (e.g., human plasma kallikrein and/or murine plasma kallikrein).

In some embodiments, the heavy chain immunoglobulin variable domain sequence comprises the heavy chain variable domain of a protein described herein, and/or the light chain immunoglobulin variable domain sequence comprises the light chain variable domain of a protein described herein.

In some embodiments, the protein comprises the heavy chain of a protein described herein, and/or the light chain of a protein described herein.

In some aspects, the disclosure features a method of treating or preventing sepsis in a subject, the method comprising:

administering an isolated protein (e.g., antibody, e.g., human antibody) comprising a heavy chain immunoglobulin variable domain sequence and a light chain immunoglobulin variable domain sequence to the subject, wherein:

the heavy chain immunoglobulin variable domain sequence comprises one, two, or three (e.g., three) CDR regions from the heavy chain variable domain of a protein described herein, and/or

the light chain immunoglobulin variable domain sequence comprises one, two, or three (e.g., three) CDR regions from the light chain variable domain of a protein described herein,

wherein the protein binds to plasma kallikrein.

In some embodiments, the plasma kallikrein binding protein does not bind prekallikrein (e.g., human prekallikrein and/or murine prekallikrein), but binds to the active form of plasma kallikrein (e.g., human plasma kallikrein and/or murine plasma kallikrein).

In some embodiments, the protein is administered in combination with another treatment for sepsis.

In some embodiments, the protein inhibits plasma kallikrein (e.g., human plasma kallikrein and/or murine plasma kallikrein).

In some embodiments, the heavy chain immunoglobulin variable domain sequence comprises the heavy chain variable domain of a protein described herein, and/or the light chain immunoglobulin variable domain sequence comprises the light chain variable domain of a protein described herein.

In some embodiments, the protein comprises the heavy chain of a protein described herein, and/or the light chain of a protein described herein.

In some aspects, the disclosure features a method of treating or preventing acute middle cerebral artery (MCA) ischemic event (stroke) in a subject, the method comprising:

administering an isolated protein (e.g., antibody, e.g., human antibody) comprising a heavy chain immunoglobulin variable domain sequence and a light chain immunoglobulin variable domain sequence to the subject, wherein:

the heavy chain immunoglobulin variable domain sequence comprises one, two, or three (e.g., three) CDR regions from the heavy chain variable domain of a protein described herein, and/or

the light chain immunoglobulin variable domain sequence comprises one, two, or three (e.g., three) CDR regions from the light chain variable domain of a protein described herein,

wherein the protein binds to plasma kallikrein.

In some embodiments, the plasma kallikrein binding protein does not bind prekallikrein (e.g., human prekallikrein and/or murine prekallikrein), but binds to the active form of plasma kallikrein (e.g., human plasma kallikrein and/or murine plasma kallikrein).

In some embodiments, the protein is administered in combination with another treatment for acute middle cerebral artery (MCA) ischemic event (stroke).

In some embodiments, the protein inhibits plasma kallikrein (e.g., human plasma kallikrein and/or murine plasma kallikrein).

In some embodiments, the heavy chain immunoglobulin variable domain sequence comprises the heavy chain variable domain of a protein described herein, and/or the light chain immunoglobulin variable domain sequence comprises the light chain variable domain of a protein described herein.

In some embodiments, the protein comprises the heavy chain of a protein described herein, and/or the light chain of a protein described herein.

In some aspects, the disclosure features a method of treating or preventing restenosis (e.g., after angioplasty) in a subject, the method comprising:

administering an isolated protein (e.g., antibody, e.g., human antibody) comprising a heavy chain immunoglobulin variable domain sequence and a light chain immunoglobulin variable domain sequence to the subject, wherein:

the heavy chain immunoglobulin variable domain sequence comprises one, two, or three (e.g., three) CDR regions from the heavy chain variable domain of a protein described herein, and/or

the light chain immunoglobulin variable domain sequence comprises one, two, or three (e.g., three) CDR regions from the light chain variable domain of a protein described herein,

wherein the protein binds to plasma kallikrein.

In some embodiments, the plasma kallikrein binding protein does not bind prekallikrein (e.g., human prekallikrein and/or murine prekallikrein), but binds to the active form of plasma kallikrein (e.g., human plasma kallikrein and/or murine plasma kallikrein).

In some embodiments, the protein is administered in combination with another treatment for restenosis (e.g., after angioplasty).

In some embodiments, the protein inhibits plasma kallikrein (e.g., human plasma kallikrein and/or murine plasma kallikrein).

In some embodiments, the heavy chain immunoglobulin variable domain sequence comprises the heavy chain variable domain of a protein described herein, and/or the light chain immunoglobulin variable domain sequence comprises the light chain variable domain of a protein described herein.

In some embodiments, the protein comprises the heavy chain of a protein described herein, and/or the light chain of a protein described herein.

In some aspects, the disclosure features a method of treating or preventing systemic lupus erythematosis nephritis in a subject, the method comprising:

administering an isolated protein (e.g., antibody, e.g., human antibody) comprising a heavy chain immunoglobulin variable domain sequence and a light chain immunoglobulin variable domain sequence to the subject, wherein:

the heavy chain immunoglobulin variable domain sequence comprises one, two, or three (e.g., three) CDR regions from the heavy chain variable domain of a protein described herein, and/or

the light chain immunoglobulin variable domain sequence comprises one, two, or three (e.g., three) CDR regions from the light chain variable domain of a protein described herein,

wherein the protein binds to plasma kallikrein.

In some embodiments, the plasma kallikrein binding protein does not bind prekallikrein (e.g., human prekallikrein and/or murine prekallikrein), but binds to the active form of plasma kallikrein (e.g., human plasma kallikrein and/or murine plasma kallikrein).

In some embodiments, the protein is administered in combination with another treatment for systemic lupus erythematosis nephritis.

In some embodiments, the protein inhibits plasma kallikrein (e.g., human plasma kallikrein and/or murine plasma kallikrein).

In some embodiments, the heavy chain immunoglobulin variable domain sequence comprises the heavy chain variable domain of a protein described herein, and/or the light chain immunoglobulin variable domain sequence comprises the light chain variable domain of a protein described herein.

In some embodiments, the protein comprises the heavy chain of a protein described herein, and/or the light chain of a protein described herein.

In some aspects, the disclosure features a method of treating or preventing burn injury in a subject, the method comprising:

administering an isolated protein (e.g., antibody, e.g., human antibody) comprising a heavy chain immunoglobulin variable domain sequence and a light chain immunoglobulin variable domain sequence to the subject, wherein:

the heavy chain immunoglobulin variable domain sequence comprises one, two, or three (e.g., three) CDR regions from the heavy chain variable domain of a protein described herein, and/or

the light chain immunoglobulin variable domain sequence comprises one, two, or three (e.g., three) CDR regions from the light chain variable domain of a protein described herein,

wherein the protein binds to plasma kallikrein.

In some embodiments, the plasma kallikrein binding protein does not bind prekallikrein (e.g., human prekallikrein and/or murine prekallikrein), but binds to the active form of plasma kallikrein (e.g., human plasma kallikrein and/or murine plasma kallikrein).

In some embodiments, the protein is administered in combination with another treatment for burn injury.

In some embodiments, the protein inhibits plasma kallikrein (e.g., human plasma kallikrein and/or murine plasma kallikrein).

In some embodiments, the heavy chain immunoglobulin variable domain sequence comprises the heavy chain variable domain of a protein described herein, and/or the light chain immunoglobulin variable domain sequence comprises the light chain variable domain of a protein described herein.

In some embodiments, the protein comprises the heavy chain of a protein described herein, and/or the light chain of a protein described herein.

In some aspects, the disclosure features a method of detecting plasma kallikrein in a sample, the method comprising: contacting the sample with a plasma kallikrein binding protein (e.g., a plasma kallikrein binding protein described herein); and detecting an interaction between the protein and the plasma kallikrein, if present.

In some embodiments, the protein includes a detectable label.

In some embodiments, the plasma kallikrein binding protein does not bind prekallikrein (e.g., human prekallikrein and/or murine prekallikrein), but binds to the active form of plasma kallikrein (e.g., human plasma kallikrein and/or murine plasma kallikrein). In some embodiments, the plasma kallikrein binding protein binds prekallikrein (e.g., human prekallikrein and/or murine prekallikrein) and the active form of plasma kallikrein (e.g., human plasma kallikrein and/or murine plasma kallikrein).

In some aspects, the disclosure features a method of detecting plasma kallikrein in a subject, the method comprising: administering a plasma kallikrein binding protein (e.g., a plasma kallikrein binding protein described herein) to a subject; and detecting an interaction between the protein and the plasma kallikrein in the subject, if present. For example, the detecting comprises imaging the subject.

In some embodiments, the protein further includes a detectable label.

In some embodiments, the plasma kallikrein binding protein does not bind prekallikrein (e.g., human prekallikrein and/or murine prekallikrein), but binds to the active form of plasma kallikrein (e.g., human plasma kallikrein and/or murine plasma kallikrein). In some embodiments, the plasma kallikrein binding protein binds prekallikrein (e.g., human prekallikrein and/or murine prekallikrein) and the active form of plasma kallikrein (e.g., human plasma kallikrein and/or murine plasma kallikrein).

In some aspects, the disclosure features a method of modulating plasma kallikrein activity, e.g., in a method of treating or preventing a plasma kallikrein associated disorder. The method includes: contacting plasma kallikrein with a plasma kallikrein binding protein (e.g., a plasma kallikrein binding protein described herein) (e.g., in a human subject), thereby modulating plasma kallikrein activity.

In some embodiments, the plasma kallikrein binding protein does not bind prekallikrein (e.g., human prekallikrein and/or murine prekallikrein), but binds to the active form of plasma kallikrein (e.g., human plasma kallikrein and/or murine plasma kallikrein).

In some embodiments, the plasma kallikrein associated disorder is selected from the group consisting of rheumatoid arthritis, gout, intestinal bowel disease, oral mucositis, neuropathic pain, inflammatory pain, spinal stenosis-degenerative spine disease, arterial or venous thrombosis, post operative ileus, aortic aneurysm, osteoarthritis, vasculitis, edema, hereditary angioedema, cerebral edema, pulmonary embolism, stroke, clotting induced by ventricular assistance devices or stents, head trauma or peri-tumor brain edema, sepsis, acute middle cerebral artery (MCA) ischemic event (stroke), restenosis (e.g., after angioplasty), systemic lupus erythematosis nephritis/vasculitis, and burn injury.

In some embodiments, the plasma kallikrein binding protein reduces abberent clotting associated with the contact activation system (i.e., intrinsic activation system) by at least 10% as measured by e.g., an APTT clotting assay. In other embodiments, the plasma kallikrein binding protein reduces abberent clotting associated with the contact activation system by at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, at least 99%, or even 100% (i.e., no detectable abberent clotting).

In some aspects, the disclosure features a method of treating a plasma kallikrein associated disorder, the method comprising administering, to a subject, a plasma kallikrein binding protein (e.g., a plasma kallikrein binding protein described herein) in an amount sufficient to treat a plasma kallikrein associated disorder in the subject. The method can further include providing to the subject a second therapy that is therapy for the plasma kallikrein associated disorder, e.g., as described herein.

In some embodiments, the plasma kallikrein associated disorder is selected from the group consisting of rheumatoid arthritis, gout, intestinal bowel disease, oral mucositis, neuropathic pain, inflammatory pain, spinal stenosis-degenerative spine disease, arterial or venous thrombosis, post operative ileus, aortic aneurysm, osteoarthritis, vasculitis, edema, hereditary angioedema, cerebral edema, pulmonary embolism, stroke, clotting induced by ventricular assistance devices or stents, head trauma or peri-tumor brain edema, sepsis, acute middle cerebral artery (MCA) ischemic event (stroke), restenosis (e.g., after angioplasty), systemic lupus erythematosis nephritis/vasculitis, and burn injury.

In some aspects, the disclosure features a method of imaging a subject. The method includes administering a plasma kallikrein binding protein (e.g., a plasma kallikrein binding protein described herein) to the subject, and e.g., detecting an interaction between the protein and the plasma kallikrein in the subject, if present.

In some embodiments, the plasma kallikrein binding protein does not bind prekallikrein (e.g., human prekallikrein and/or murine prekallikrein), but binds to the active form of plasma kallikrein (e.g., human plasma kallikrein and/or murine plasma kallikrein). In some embodiments, the plasma kallikrein binding protein binds prekallikrein (e.g., human prekallikrein and/or murine prekallikrein) and the active form of plasma kallikrein (e.g., human plasma kallikrein and/or murine plasma kallikrein).

In some embodiments, the protein does not inhibit plasma kallikrein activity.

In some embodiments, the protein inhibits plasma kallikrein activity (e.g., human plasma kallikrein and/or murine plasma kallikrein).

In some embodiments, the plasma kallikrein binding protein may include a detectable label (e.g., a radionuclide or an MRI-detectable label).

In some embodiments, the subject has or is suspected of having a plasma kallikrein associated disorder. The method is useful, e.g., for diagnosis of a plasma kallikrein associated disorder.

In some embodiments, the plasma kallikrein associated disorder is selected from the group consisting of rheumatoid arthritis, gout, intestinal bowel disease, oral mucositis, neuropathic pain, inflammatory pain, spinal stenosis-degenerative spine disease, arterial or venous thrombosis, post operative ileus, aortic aneurysm, osteoarthritis, vasculitis, edema, hereditary angioedema, cerebral edema, pulmonary embolism, stroke, clotting induced by ventricular assistance devices or stents, head trauma or peri-tumor brain edema, sepsis, acute middle cerebral artery (MCA) ischemic event (stroke), restenosis (e.g., after angioplasty), systemic lupus erythematosis nephritis, and burn injury.

In some embodiments, the plasma kallikrein binding protein reduces abberent clotting associated with the contact activation system (i.e., intrinsic activation system) by at least 10% as measured by e.g., an APTT clotting assay (e.g., by at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, at least 99%, or even 100% (i.e., no detectable abberent clotting)).

In some aspects, the disclosure features a method of imaging plasma kallikrein, e.g., in a subject or sample (e.g., biopsy sample). The method includes administering a plasma kallikrein binding protein (e.g., a plasma kallikrein binding protein described herein), e.g., to the subject or the sample, and detecting an interaction between the protein and the plasma kallikrein, if present.

In some embodiments, the plasma kallikrein binding protein does not bind prekallikrein (e.g., human prekallikrein and/or murine prekallikrein), but binds to the active form of plasma kallikrein (e.g., human plasma kallikrein and/or murine plasma kallikrein). In some embodiments, the plasma kallikrein binding protein binds prekallikrein (e.g., human prekallikrein and/or murine prekallikrein) and the active form of plasma kallikrein (e.g., human plasma kallikrein and/or murine plasma kallikrein).

In some embodiments, the protein does not inhibit plasma kallikrein activity.

In some embodiments, the protein inhibits plasma kallikrein activity (e.g., human plasma kallikrein and/or murine plasma kallikrein).

In some embodiments, the plasma kallikrein binding protein may include a detectable label (e.g., a radionuclide or an MRI-detectable label).

In some embodiments, the subject has or is suspected of having a plasma kallikrein associated disorder. The method is useful, e.g., for diagnosis of a plasma kallikrein associated disorder.

In some embodiments, the plasma kallikrein associated disorder is selected from the group consisting of rheumatoid arthritis, gout, intestinal bowel disease, oral mucositis, neuropathic pain, inflammatory pain, spinal stenosis-degenerative spine disease, arterial or venous thrombosis, post operative ileus, aortic aneurysm, osteoarthritis, vasculitis, edema, hereditary angioedema, cerebral edema, pulmonary embolism, stroke, clotting induced by ventricular assistance devices or stents, head trauma or peri-tumor brain edema, sepsis, acute middle cerebral artery (MCA) ischemic event (stroke), restenosis (e.g., after angioplasty), systemic lupus erythematosis nephritis, and burn injury.

In some embodiments, the plasma kallikrein binding protein reduces abberent clotting associated with the contact activation system (i.e., intrinsic activation system) by at least 10% as measured by e.g., an APTT clotting assay (e.g., by at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, at least 99%, or even 100% (i.e., no detectable abberent clotting)).

In one aspect, the disclosure features the use of a plasma kallikrein binding protein described herein for the treatment of a disorder described herein, e.g., rheumatoid arthritis, gout, intestinal bowel disease, oral mucositis, neuropathic pain, inflammatory pain, spinal stenosis-degenerative spine disease, arterial or venous thrombosis, post operative ileus, aortic aneurysm, osteoarthritis, vasculitis, edema, hereditary angioedema, cerebral edema, pulmonary embolism, stroke, clotting induced by ventricular assistance devices or stents, head trauma or peri-tumor brain edema, sepsis, acute middle cerebral artery (MCA) ischemic event (stroke), restenosis (e.g., after angioplasty), systemic lupus erythematosis nephritis, or burn injury; or to promote wound healing.

In some embodiments, the plasma kallikrein binding protein does not bind prekallikrein (e.g., human prekallikrein and/or murine prekallikrein), but binds to the active form of plasma kallikrein (e.g., human plasma kallikrein and/or murine plasma kallikrein). In some embodiments, the plasma kallikrein binding protein binds prekallikrein (e.g., human prekallikrein and/or murine prekallikrein) and the active form of plasma kallikrein (e.g., human plasma kallikrein and/or murine plasma kallikrein).

In one aspect, the disclosure features the use of a plasma kallikrein binding protein described herein for the manufacture of a medicament for the treatment of a disorder described herein, e.g., rheumatoid arthritis, gout, intestinal bowel disease, oral mucositis, neuropathic pain, inflammatory pain, spinal stenosis-degenerative spine disease, arterial or venous thrombosis, post operative ileus, aortic aneurysm, osteoarthritis, vasculitis, edema, hereditary angioedema, cerebral edema, pulmonary embolism, stroke, clotting induced by ventricular assistance devices or stents, head trauma or peri-tumor brain edema, sepsis, acute middle cerebral artery (MCA) ischemic event (stroke), restenosis (e.g., after angioplasty), systemic lupus erythematosis nephritis, or burn injury; or for the manufacture of a medicament for wound healing.

In some embodiments, the plasma kallikrein binding protein reduces abberent clotting associated with the contact activation system (i.e., intrinsic activation system) by at least 10% as measured by e.g., an APTT clotting assay (e.g., by at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, at least 99%, or even 100% (i.e., no detectable abberent clotting)).

In some embodiments, the plasma kallikrein binding protein does not bind prekallikrein (e.g., human prekallikrein and/or murine prekallikrein), but binds to the active form of plasma kallikrein (e.g., human plasma kallikrein and/or murine plasma kallikrein).

The details of one or more embodiments of the invention are set forth in the accompanying drawings and the description below. Other features, objects, and advantages of the invention will be apparent from the description and drawings, and from the claims.

The contents of all cited references including literature references, issued patents, published or non-published patent applications cited throughout this application as well as those listed below are hereby expressly incorporated by reference in their entireties. In case of conflict, the present application, including any definitions herein, will control.

BRIEF DESCRIPTION OF DRAWINGS

FIG. 1 is a schematic representation of the role of plasma kallikrein (pKal) in intrinsic coagulation pathway and inflammation.

FIG. 2 depicts the effect of M162-A04 on carrageenan-induced rat paw edema. Paw swelling was measured by water displacement.

FIG. 3 depicts the effect of M162-A04 on carrageenan-induced thermal hyperalgesia. Pain latency was measured by the Hargreaves method after carrageenan injection.

FIG. 4 depicts the alignment of the light chain DNA sequence of nongermlined (X63-G06) and germlined, codon optimized (X81-B01) versions of the same antibody discovered using ROLIC affinity maturation. Positions indicated with an asterisk (*) are conserved, whereas blank spaces correspond to bases changed in X81-B01 due to either codon optimization or germlining.

FIG. 5 depicts the alignment of the light chain amino acid sequence of nongermlined (X63-G06) and germlined, codon optimized (X81-B01) versions of the same antibody discovered using ROLIC affinity maturation. Positions indicated with an asterisk (*) are conserved, whereas blank spaces correspond to amino acids changed in X81-B01 due to germlining. A total of 11 amino acids differ between the nongermlined (X63-G06) and germlined, codon optimized antibody (X81-B01).

FIG. 6 depicts the alignment of the heavy chain DNA sequence of nongermlined (X63-G06) and germlined, codon optimized (X81-B01) versions of the same antibody discovered using ROLIC affinity maturation. Positions indicated with an asterisk (*) are conserved, whereas blank spaces correspond to DNA bases changed in X81-B01 due to codon optimization.

FIG. 7 depicts the alignment of the heavy chain amino acid sequence of nongermlined (X63-G06) and germlined, codon optimized (X81-B01) versions of the same antibody discovered using ROLIC affinity maturation. Positions indicated with an asterisk (*) are conserved. The two antibodies have the same amino acid sequence in the heavy chain.

FIG. 8A depicts the EPI-KAL2 competition for X81-B01 binding pKal. X81-B01 (IgG) was captured on an anti-human Fc fragment specific surface of a CM5 BIACORE® chip. pKal (100 nM) was flowed over the surface in the presence (lower sensorgram in the figure) or absence of 1 μM EPI-KAL2 (upper sensorgram in the figure).

FIG. 8B depicts the EPI-KAL2 competition for X67-D03 binding pKal. X67-D03 (IgG) was captured on an anti-human Fc fragment specific surface of a CM5 Biacore chip. pKal (100 nM) was flowed over the surface in the presence (lower sensorgram in the figure) or absence of 1 μM EPI-KAL2 (upper sensorgram in the figure).

FIG. 9 depicts the results of CLIPS epitope mapping for antibodies listed in Table 12.

FIGS. 10A-10C depict ClustalW alignment of pKal sequences from different species. Positions indicated by a "*" are conserved positions between, whereas positions indicated ":" indicate conservative substitutions between species. Positions indicated by a "." have nonconservative substitutions in some species. Stretches of amino acids indicated by the symbol "@" were shown to be highly solvent exposed by solvent accessible surface area calculation. Stretches of amino acids indicated by a "+" were identified as potential epitopes of antibodies listed in Table 12. Amino acids highlighted in grey were found by solvent accessible surface area calculation to be buried when complexed with a Kunitz domain active site inhibitor. The underlined positions are the amino acids that form the catalytic triad (His434, Asp483, and Ser578, numbering based on the human sequence).

FIGS. 11A and 11B depict a Biacore competition analysis with epi-kal2, as described herein in Example 12, for (i) DX-2922, and (ii) M6-D09 antibodies.

FIG. 12 depicts a Biacore competition analysis with AEBSF, as described herein in Example 12, for (i) DX-2911, and (ii) M6-D09 antibodies.

FIG. 13 depicts a Biocore analysis showing that DX-2922 binds to plasma kallikrein that bound to high molecular weight kininogen (HMWK).

FIG. 14 depicts a graph showing dose dependent inhibition of edema by X101-A01 in carrageenan-induced paw edema (CPE) in rats.

FIG. 15 depicts a graph showing dose dependent inhibition of edema by intraperitoneal administration DX-2930 in carrageenan-induced paw edema in the rat.

FIG. 16 depicts a graph showing dose dependent inhibition of edema by subcutaneous administration DX-2930 in carrageenan-induced paw edema in the rat.

FIG. 17 depicts a graph showing mean DX-2930 serum concentrations following IV and SC administration to Sprague-Dawley rats for pharmacokinetic assessments.

FIG. 18 depicts a graph showing mean DX-2930 serum concentrations following IV and SC administration to cynomolgus monkeys for pharmacokinetic assessments.

DETAILED DESCRIPTION

Definitions

For convenience, before further description of the present invention, certain terms employed in the specification, examples and appended claims are defined here. Other terms are defined as they appear in the specification.

The singular forms "a", "an", and "the" include plural references unless the context clearly dictates otherwise.

The term "agonist," as used herein, is meant to refer to an agent that mimics or up-regulates (e.g., potentiates or supplements) the bioactivity of a protein. An agonist can be a wild-type protein or derivative thereof having at least one bioactivity of the wild-type protein. An agonist can also be a compound which increases at least one bioactivity of a protein. An agonist can also be a compound which increases the interaction of a polypeptide with another molecule, e.g., a target peptide or nucleic acid.

"Antagonist" as used herein is meant to refer to an agent that downregulates (e.g., suppresses or inhibits) at least one bioactivity of a protein. An antagonist can be a compound which inhibits or decreases the interaction between a protein and another molecule, e.g., a target peptide or enzyme substrate. An antagonist can also be a compound which reduces the amount of expressed protein present.

The term "antibody" refers to a protein that includes at least one immunoglobulin variable domain (variable region) or immunoglobulin variable domain (variable region) sequence. For example, an antibody can include a heavy (H) chain variable region (abbreviated herein as VH or HV), and a light (L) chain variable region (abbreviated herein as VL or LV). In another example, an antibody includes two heavy (H) chain variable regions and two light (L) chain variable regions. The term "antibody" encompasses antigen-binding fragments of antibodies (e.g., single chain antibodies, Fab and sFab fragments, $F(ab')_2$, Fd fragments, Fv fragments, scFv, and domain antibodies (dAb) fragments (de Wildt et al., Eur J Immunol. 1996; 26(3):629-39)) as well as complete antibodies. An antibody can have the structural features of IgA, IgG, IgE, IgD, IgM (as well as subtypes thereof). Antibodies may be from any source, but primate (human and non-human primate) and primatized are preferred.

The VH and VL regions can be further subdivided into regions of hypervariability, termed "complementarity determining regions" ("CDRs"), interspersed with regions that are more conserved, termed "framework regions" ("FRs"). The extent of the framework region and CDRs have been defined (see, Kabat, E. A., et al. (1991) Sequences of Proteins of Immunological Interest, Fifth Edition, U.S. Department of Health and Human Services, NIH Publication No. 91-3242, and Chothia, C. et al. (1987) J. Mol. Biol. 196:901-917). Kabat definitions are used herein. Each VH and VL is typically composed of three CDRs and four FRs, arranged from amino-terminus to carboxy-terminus in the following order: FR1, CDR1, FR2, CDR2, FR3, CDR3, FR4.

As used herein, an "immunoglobulin variable domain sequence" refers to an amino acid sequence which can form the structure of an immunoglobulin variable domain such that one or more CDR regions are positioned in a conformation suitable for an antigen binding site. For example, the sequence may include all or part of the amino acid sequence of a naturally-occurring variable domain. For example, the sequence may omit one, two or more N- or C-terminal amino acids, internal amino acids, may include one or more insertions or additional terminal amino acids, or may include other alterations. In one embodiment, a polypeptide that includes immunoglobulin variable domain sequence can associate with another immunoglobulin variable domain sequence to form an antigen binding site, e.g., a structure that preferentially interacts with plasma kallikrein.

The VH or VL chain of the antibody can further include all or part of a heavy or light chain constant region, to thereby form a heavy or light immunoglobulin chain, respectively. In one embodiment, the antibody is a tetramer of two heavy immunoglobulin chains and two light immunoglobulin chains, wherein the heavy and light immunoglobulin chains are inter-connected by, e.g., disulfide bonds. In IgGs, the heavy chain constant region includes three immunoglobulin domains, CH1, CH2 and CH3. The light chain constant region includes a CL domain. The variable region of the heavy and light chains contains a binding domain that interacts with an antigen. The constant regions of the antibodies typically mediate the binding of the antibody to host tissues or factors, including various cells of the immune system (e.g., effector cells) and the first component (C1q) of the classical complement system. The light chains of the immunoglobulin may be of types kappa or lambda. In one embodiment, the antibody is glycosylated. An antibody can be functional for antibody-dependent cytotoxicity and/or complement-mediated cytotoxicity.

One or more regions of an antibody can be human or effectively human. For example, one or more of the variable regions can be human or effectively human. For example, one or more of the CDRs can be human, e.g., HC CDR1, HC CDR2, HC CDR3, LC CDR1, LC CDR2, and/or LC CDR3. Each of the light chain (LC) and/or heavy chain (HC) CDRs can be human. HC CDR3 can be human. One or more of the framework regions can be human, e.g., FR1, FR2, FR3, and/or FR4 of the HC and/or LC. For example, the Fc region can be human. In one embodiment, all the framework regions are human, e.g., derived from a human somatic cell, e.g., a hematopoietic cell that produces immunoglobulins or a non-hematopoietic cell. In one embodiment, the human sequences are germline sequences, e.g., encoded by a germline nucleic acid. In one embodiment, the framework (FR) residues of a selected Fab can be converted to the amino-acid type of the corresponding residue in the most similar primate germline gene, especially the human germline gene. One or more of the constant regions can be human or effectively human. For example, at least 70, 75, 80, 85, 90, 92, 95, 98, or 100% of an immunoglobulin variable domain, the constant region, the constant domains (CH1, CH2, CH3, and/or CL1), or the entire antibody can be human or effectively human.

All or part of an antibody can be encoded by an immunoglobulin gene or a segment thereof. Exemplary human immunoglobulin genes include the kappa, lambda, alpha (IgA1 and IgA2), gamma (IgG1, IgG2, IgG3, IgG4), delta, epsilon and mu constant region genes, as well as the many immunoglobulin variable region genes. Full-length immunoglobulin "light chains" (about 25 KDa or about 214 amino acids) are encoded by a variable region gene at the NH2-terminus (about 110 amino acids) and a kappa or lambda constant region gene at the COOH-terminus. Full-length immunoglobulin "heavy chains" (about 50 KDa or about 446 amino acids), are similarly encoded by a variable region gene (about 116 amino acids) and one of the other aforementioned constant region genes, e.g., gamma (encoding about 330 amino acids). The length of human HC varies considerably because HC CDR3 varies from about 3 amino-acid residues to over 35 amino-acid residues.

The term "antigen-binding fragment" of a full length antibody refers to one or more fragments of a full-length antibody that retain the ability to specifically bind to a target of interest. Examples of binding fragments encompassed within the term "antigen-binding fragment" of a full length antibody and that retain functionality include (i) a Fab fragment, a monovalent fragment consisting of the VL, VH, CL and CH1 domains; (ii) a $F(ab')_2$ fragment, a bivalent fragment including two Fab fragments linked by a disulfide bridge at the hinge region; (iii) a Fd fragment consisting of the VH and CH1 domains; (iv) a Fv fragment consisting of the VL and VH domains of a single arm of an antibody, (v) a dAb fragment (Ward et al., (1989) Nature 341:544-546), which consists of a VH domain; and (vi) an isolated complementarity determining region (CDR). Furthermore, although the two domains of the Fv fragment, VL and VH, are coded for by separate genes, they can be joined, using recombinant methods, by a synthetic linker that enables them to be made as a single protein chain in which the VL and VH regions pair to form monovalent molecules known as single chain Fv (scFv). See e.g., U.S. Pat. Nos. 5,260,203, 4,946,778, and 4,881,175; Bird et al. (1988) Science 242:423-426; and Huston et al. (1988) Proc. Natl. Acad. Sci. USA 85:5879-5883.

Antibody fragments can be obtained using any appropriate technique including conventional techniques known to those with skill in the art. The term "monospecific antibody" refers to an antibody that displays a single binding specificity and affinity for a particular target, e.g., epitope. This term includes a "monoclonal antibody" or "monoclonal antibody composition," which as used herein refers to a preparation of antibodies or fragments thereof of single molecular composition, irrespective of how the antibody was generated.

Antibodies are "germlined" by reverting one or more non-germline amino acids in framework regions to corresponding germline amino acids of the antibody, so long as binding properties are substantially retained.

The inhibition constant (Ki) provides a measure of inhibitor potency; it is the concentration of inhibitor required to reduce enzyme activity by half and is not dependent on enzyme or substrate concentrations. The apparent Ki ($K_{i,app}$) is obtained at different substrate concentrations by measuring the inhibitory effect of different concentrations of inhibitor (e.g., inhibitory binding protein) on the extent of the reaction (e.g., enzyme activity); fitting the change in pseudo-first order rate constant as a function of inhibitor concentration to the Morrison equation (Equation 1) yields an estimate of the apparent Ki value. The Ki is obtained from the y-intercept extracted from a linear regression analysis of a plot of Ki,app versus substrate concentration.

$$v = v_o - v_o\left(\frac{(K_{i,app} + I + E) - \sqrt{(K_{i,app} + I + E)^2 - 4 \cdot I \cdot E}}{2 \cdot E}\right) \quad \text{Equation 1}$$

Where v=measured velocity; v_0=velocity in the absence of inhibitor; $K_{i,app}$=apparent inhibition constant; I=total inhibitor concentration; and E=total enzyme concentration.

As used herein, "binding affinity" refers to the apparent association constant or K_A. The K_A is the reciprocal of the dissociation constant (K_D). A binding protein may, for example, have a binding affinity of at least 10^5, 10^6, 10^7, 10^8, 10^9, 10^{10} and 10^{11} M^{-1} for a particular target molecule, e.g., plasma kallikrein. Higher affinity binding of a binding protein to a first target relative to a second target can be indicated by a higher K_A (or a smaller numerical value K_D) for binding the first target than the K_A (or numerical value K_D) for binding the second target. In such cases, the binding protein has specificity for the first target (e.g., a protein in a first conformation or mimic thereof) relative to the second target (e.g., the same protein in a second conformation or mimic thereof; or a second protein). Differences in binding affinity (e.g., for specificity or other comparisons) can be at least 1.5, 2, 3, 4, 5, 10, 15, 20, 37.5, 50, 70, 80, 91, 100, 500, 1000, 10,000 or 10^5 fold.

Binding affinity can be determined by a variety of methods including equilibrium dialysis, equilibrium binding, gel filtration, ELISA, surface plasmon resonance, or spectroscopy (e.g., using a fluorescence assay). Exemplary conditions for evaluating binding affinity are in HBS-P buffer (10 mM HEPES pH7.4, 150 mM NaCl, 0.005% (v/v) Surfactant P20). These techniques can be used to measure the concentration of bound and free binding protein as a function of binding protein (or target) concentration. The concentration of bound binding protein ([Bound]) is related to the concentration of free binding protein ([Free]) and the concentration of binding sites for the binding protein on the target where (N) is the number of binding sites per target molecule by the following equation:

$$[\text{Bound}] = N \cdot [\text{Free}]/((1/K_A) + [\text{Free}]).$$

It is not always necessary to make an exact determination of K_A, though, since sometimes it is sufficient to obtain a quantitative measurement of affinity, e.g., determined using a method such as ELISA or FACS analysis, is proportional to K_A, and thus can be used for comparisons, such as determining whether a higher affinity is, e.g., 2-fold higher, to obtain a qualitative measurement of affinity, or to obtain an inference of affinity, e.g., by activity in a functional assay, e.g., an in vitro or in vivo assay.

The term "binding protein" refers to a protein that can interact with a target molecule. This term is used interchangeably with "ligand." A "plasma kallikrein binding protein" refers to a protein that can interact with (e.g., bind) plasma kallikrein, and includes, in particular, proteins that preferentially or specifically interact with and/or inhibit plasma kallikrein. A protein inhibits plasma kallikrein if it causes a decrease in the activity of plasma kallikrein as compared to the activity of plasma kallikrein in the absence of the protein and under the same conditions. In some embodiments, the plasma kallikrein binding protein is an antibody.

A "conservative amino acid substitution" is one in which the amino acid residue is replaced with an amino acid residue having a similar side chain. Families of amino acid residues having similar side chains have been defined in the art. These families include amino acids with basic side chains (e.g., lysine, arginine, histidine), acidic side chains (e.g., aspartic acid, glutamic acid), uncharged polar side chains (e.g., glycine, asparagine, glutamine, serine, threonine, tyrosine, cysteine), nonpolar side chains (e.g., alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine, tryptophan), beta-branched side chains (e.g., threonine, valine, isoleucine) and aromatic side chains (e.g., tyrosine, phenylalanine, tryptophan, histidine).

It is possible for one or more framework and/or CDR amino acid residues of a binding protein to include one or more mutations (e.g., substitutions (e.g., conservative substitutions or substitutions of non-essential amino acids), insertions, or deletions) relative to a binding protein described herein. A plasma kallikrein binding protein may have mutations (e.g., substitutions (e.g., conservative substitutions or substitutions of non-essential amino acids), insertions, or deletions) (e.g., at least one, two, three, or four, and/or less than 15, 12, 10, 9, 8, 7, 6, 5, 4, 3, or 2 mutations) relative to a binding protein described herein, e.g., mutations which do not have a substantial effect on protein function. The mutations can be present in framework regions, CDRs, and/or constant regions. In some embodiments, the mutations are present in a framework region. In some embodiments, the mutations are present in a CDR. In some embodiments, the mutations are present in a constant region. Whether or not a particular substitution will be tolerated, i.e., will not adversely affect biological properties, such as binding activity, can be predicted, e.g., by evaluating whether the mutation is conservative or by the method of Bowie, et al. (1990) Science 247:1306-1310.

Motif sequences for biopolymers can include positions which can be varied amino acids. For example, the symbol "X" in such a context generally refers to any amino acid (e.g., any of the twenty natural amino acids) unless otherwise specified, e.g., to refer to any non-cysteine amino acid. Other allowed amino acids can also be indicated for example, using parentheses and slashes. For example, "(A/W/F/N/Q)" means that alanine, tryptophan, phenylalanine, asparagine, and glutamine are allowed at that particular position.

An "effectively human" immunoglobulin variable region is an immunoglobulin variable region that includes a sufficient number of human framework amino acid positions such that the immunoglobulin variable region does not elicit an immunogenic response in a normal human. An "effectively human" antibody is an antibody that includes a sufficient number of human amino acid positions such that the antibody does not elicit an immunogenic response in a normal human.

An "epitope" refers to the site on a target compound that is bound by a binding protein (e.g., an antibody such as a Fab or full length antibody). In the case where the target compound is a protein, the site can be entirely composed of amino acid components, entirely composed of chemical modifications of amino acids of the protein (e.g., glycosyl moieties), or composed of combinations thereof. Overlapping epitopes include at least one common amino acid residue, glycosyl group, phosphate group, sulfate group, or other molecular feature.

A first binding protein (e.g., antibody) "binds to the same epitope" as a second binding protein (e.g., antibody) if the first binding protein binds to the same site on a target compound that the second binding protein binds, or binds to a site that overlaps (e.g., 50%, 60%, 70%, 80%, 90%, or 100% overlap, e.g., in terms of amino acid sequence or other molecular feature (e.g., glycosyl group, phosphate group, or sulfate group)) with the site that the second binding protein binds.

A first binding protein (e.g., antibody) "competes for binding" with a second binding protein (e.g., antibody) if the binding of the first binding protein to its epitope decreases (e.g., by 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%, or more) the amount of the second binding protein that binds to its epitope. The competition can be direct (e.g., the first binding protein binds to an epitope that is the same as, or overlaps with, the epitope bound by the second binding protein), or indirect (e.g., the binding of the first binding protein to its epitope causes a steric change in the target compound that decreases the ability of the second binding protein to bind to its epitope).

Calculations of "homology" or "sequence identity" between two sequences (the terms are used interchangeably herein) are performed as follows. The sequences are aligned for optimal comparison purposes (e.g., gaps can be introduced in one or both of a first and a second amino acid or nucleic acid sequence for optimal alignment and non-homologous sequences can be disregarded for comparison purposes). The optimal alignment is determined as the best score using the GAP program in the GCG software package with a Blossum 62 scoring matrix with a gap penalty of 12, a gap extend penalty of 4, and a frameshift gap penalty of 5. The amino acid residues or nucleotides at corresponding amino acid positions or nucleotide positions are then compared. When a position in the first sequence is occupied by the same amino acid residue or nucleotide as the corresponding position in the second sequence, then the molecules are identical at that position (as used herein amino acid or nucleic acid "identity" is equivalent to amino acid or nucleic acid "homology"). The percent identity between the two sequences is a function of the number of identical positions shared by the sequences.

In a preferred embodiment, the length of a reference sequence aligned for comparison purposes is at least 30%, preferably at least 40%, more preferably at least 50%, even more preferably at least 60%, and even more preferably at least 70%, 80%, 90%, 92%, 95%, 97%, 98%, or 100% of the length of the reference sequence. For example, the reference sequence may be the length of the immunoglobulin variable domain sequence.

A "humanized" immunoglobulin variable region is an immunoglobulin variable region that is modified to include a sufficient number of human framework amino acid positions such that the immunoglobulin variable region does not elicit an immunogenic response in a normal human Descriptions of "humanized" immunoglobulins include, for example, U.S. Pat. No. 6,407,213 and U.S. Pat. No. 5,693,762.

As used herein, the term "hybridizes under low stringency, medium stringency, high stringency, or very high stringency conditions" describes conditions for hybridization and washing. Guidance for performing hybridization reactions can be found in Current Protocols in Molecular Biology, John Wiley & Sons, N.Y. (1989), 6.3.1-6.3.6. Aqueous and nonaqueous methods are described in that reference and either can be used. Specific hybridization conditions referred to herein are as follows: (1) low stringency hybridization conditions in 6× sodium chloride/sodium citrate (SSC) at about 45° C., followed by two washes in 0.2×SSC, 0.1% SDS at least at 50° C. (the temperature of the washes can be increased to 55° C. for low stringency conditions); (2) medium stringency hybridization conditions in 6×SSC at about 45° C., followed by one or more washes in 0.2×SSC, 0.1% SDS at 60° C.; (3) high stringency hybridization conditions in 6×SSC at about 45° C., followed by one or more washes in 0.2×SSC, 0.1% SDS at 65° C.; and (4) very high stringency hybridization conditions are 0.5M sodium phosphate, 7% SDS at 65° C., followed by one or more washes at 0.2×SSC, 1% SDS at 65° C. Very high stringency conditions (4) are the preferred conditions and the ones that should be used unless otherwise specified. The disclosure includes nucleic acids that hybridize with low, medium, high, or very high stringency to a nucleic acid described herein or to a complement thereof, e.g., nucleic acids encoding a binding protein described herein. The nucleic acids can be the same length or within 30, 20, or 10% of the length of the reference nucleic acid. The nucleic acid can correspond to a region encoding an immunoglobulin variable domain sequence described herein.

An "isolated composition" refers to a composition that is removed from at least 90% of at least one component of a natural sample from which the isolated composition can be obtained. Compositions produced artificially or naturally can be "compositions of at least" a certain degree of purity if the species or population of species of interest is at least 5, 10, 25, 50, 75, 80, 90, 92, 95, 98, or 99% pure on a weight-weight basis.

An "isolated" protein refers to a protein that is removed from at least 90% of at least one component of a natural sample from which the isolated protein can be obtained. Proteins can be "of at least" a certain degree of purity if the species or population of species of interest is at least 5, 10, 25, 50, 75, 80, 90, 92, 95, 98, or 99% pure on a weight-weight basis.

The term "modulator" refers to a polypeptide, nucleic acid, macromolecule, complex, molecule, small molecule, compound, species or the like (naturally-occurring or non-naturally-occurring), or an extract made from biological materials such as bacteria, plants, fungi, or animal cells or tissues, that may be capable of causing modulation. Modulators may be evaluated for potential activity as inhibitors or activators (directly or indirectly) of a functional property, biological activity or process, or combination of them, (e.g., agonist, partial antagonist, partial agonist, inverse agonist, antagonist, anti-microbial agents, inhibitors of microbial infection or proliferation, and the like) by inclusion in assays. In such assays, many modulators may be screened at one time. The activity of a modulator may be known, unknown or partially known.

A "non-essential" amino acid residue is a residue that can be altered from the wild-type sequence of the binding agent, e.g., the antibody, without abolishing or more preferably, without substantially altering a biological activity, whereas changing an "essential" amino acid residue results in a substantial loss of activity.

A "patient," "subject" or "host" (these terms are used interchangeably) to be treated by the subject method may mean either a human or non-human animal.

The terms "prekallikrein" and "preplasma kallikrein" are used interchangeably herein and refer to the zymogen form of active plasma kallikrein, which is also known as prekallikrein.

The term "preventing" or to "prevent" a disease in a subject refers to subjecting the subject to a pharmaceutical treatment, e.g., the administration of a drug, such that at least one symptom of the disease is prevented, that is, administered prior to clinical manifestation of the unwanted condition (e.g., disease or other unwanted state of the host animal) so that it protects the host against developing the unwanted condition. "Preventing" a disease may also be referred to as "prophylaxis" or "prophylactic treatment."

As used herein, the term "substantially identical" (or "substantially homologous") is used herein to refer to a first amino acid or nucleic acid sequence that contains a sufficient number of identical or equivalent (e.g., with a similar side chain, e.g., conserved amino acid substitutions) amino acid residues or nucleotides to a second amino acid or nucleic acid sequence such that the first and second amino acid or nucleic acid sequences have (or encode proteins having) similar activities, e.g., a binding activity, a binding preference, or a biological activity. In the case of antibodies, the second antibody has the same specificity and has at least 50%, at least 25%, or at least 10% of the affinity relative to the same antigen.

Sequences similar or homologous (e.g., at least about 85% sequence identity) to the sequences disclosed herein are also part of this application. In some embodiments, the sequence identity can be about 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or higher. In some embodiments, a plasma kallikrein binding protein can have about 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or higher sequence identity to a binding protein described herein. In some embodiments, a plasma kallikrein binding protein can have about 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or higher sequence identity in the HC and/or LC framework regions (e.g., HC and/or LC FR 1, 2, 3, and/or 4) to a binding protein described herein. In some embodiments, a plasma kallikrein binding protein can have about 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or higher sequence identity in the HC and/or LC CDRs (e.g., HC and/or LC CDR1, 2, and/or 3) to a binding protein described herein. In some embodiments, a plasma kallikrein binding protein can have about 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or higher sequence identity in the constant region (e.g., CH1, CH2, CH3, and/or CL1) to a binding protein described herein.

In addition, substantial identity exists when the nucleic acid segments hybridize under selective hybridization conditions (e.g., highly stringent hybridization conditions), to the complement of the strand. The nucleic acids may be present in whole cells, in a cell lysate, or in a partially purified or substantially pure form.

Statistical significance can be determined by any art known method. Exemplary statistical tests include: the Students T-test, Mann Whitney U non-parametric test, and Wilcoxon non-parametric statistical test. Some statistically significant relationships have a P value of less than 0.05 or 0.02. Particular binding proteins may show a difference, e.g., in specificity or binding that are statistically significant (e.g., P value<0.05 or 0.02). The terms "induce", "inhibit", "potentiate", "elevate", "increase", "decrease" or the like, e.g., which denote distinguishable qualitative or quantitative differences between two states, may refer to a difference, e.g., a statistically significant difference, between the two states.

A "therapeutically effective dosage" preferably modulates a measurable parameter, e.g., plasma kallikrein activity, by a statistically significant degree or at least about 20%, more preferably by at least about 40%, even more preferably by at least about 60%, and still more preferably by at least about 80% relative to untreated subjects. The ability of a compound to modulate a measurable parameter, e.g., a disease-associated parameter, can be evaluated in an animal model system predictive of efficacy in human disorders and conditions, e.g., rheumatoid arthritis or oral mucositis. Alternatively, this property of a composition can be evaluated by examining the ability of the compound to modulate a parameter in vitro.

"Treating" a disease (or condition) in a subject or "treating" a subject having a disease refers to subjecting the subject to a pharmaceutical treatment, e.g., the administration of a drug, such that at least one symptom of the disease is cured, alleviated or decreased.

The term "preventing" a disease in a subject refers to subjecting the subject to a pharmaceutical treatment, e.g., the administration of a drug, such that at least one symptom of the disease is prevented, that is, administered prior to clinical manifestation of the unwanted condition (e.g., disease or other unwanted state of the host animal) so that it protects the host against developing the unwanted condition. "Preventing" a disease may also be referred to as "prophylaxis" or "prophylactic treatment."

A "prophylactically effective amount" refers to an amount effective, at dosages and for periods of time necessary, to achieve the desired prophylactic result. Typically, because a prophylactic dose is used in subjects prior to or at an earlier stage of disease, the prophylactically effective amount will be less than the therapeutically effective amount.

As used herein the term "DX-2922" as used inchangeably with the term "X101-A01". Other variants of this antibody are described below.

Antibody Identification	Description
X63-G06	Non-germlined Fab discovered using ROLIC, same HC but different LC as M160-G12
X81-B01	Germlined IgG produced in HEK 293T cells
X101-A01	Germlined IgG produced in CHO cells, same HC and LC sequence as X81-B01
DX-2922	Alternate nomenclature for X101-A01

As used herein the term "DX-2930" as used inchangeably with the term "X124-G01". Other variants of this antibody are described below.

Antibody Identification	Description
M162-A04	Non-germlined Fab discovered using phage display
M199-A08	Heavy chain CDR3 varied Fab derived by affinity maturation of M162-A04
X115-F02	Germlined Fab produced in 293T cells, same variable heavy chain as X124-G01
X124-G01 or DX-2930	Germlined IgG produced in CHO cells, same variable heavy chain as X115-F02, same variable LC as X115-F02 except C-terminal Lys is removed

As used herein the term "unstructured recombinant polymer" (URP) refers to an amino acid sequence that lacks a secondary structure and shares commonality with denatured peptide sequences, e.g., exhibiting a typical behavior like denatured peptide sequences, under physiological conditions. URP sequences lack a defined tertiary structure and they have limited or no secondary structure as detected by, e.g., Chou-Fasman algorithm.

Plasma Kallikrein Binding Proteins

Plasma kallikrein binding proteins can be full-length (e.g., an IgG (e.g., an IgG1, IgG2, IgG3, IgG4), IgM, IgA (e.g., IgA1, IgA2), IgD, and IgE) or can include only an antigen-binding fragment (e.g., a Fab, F(ab')2 or scFv fragment. The binding protein can include two heavy chain immunoglobulins and two light chain immunoglobulins, or can be a single chain antibody. Plasma kallikrein binding proteins can be recombinant proteins such as humanized, CDR grafted, chimeric, deimmunized, or in vitro generated antibodies, and may optionally include constant regions derived from human germline immunoglobulin sequences. In one embodiment, the plasma kallikrein binding protein is a monoclonal antibody.

In one aspect, the disclosure features a protein (e.g., an isolated protein) that binds to plasma kallikrein (e.g., human plasma kallikrein and/or murine kallikrein) and includes at least one immunoglobulin variable region. For example, the protein includes a heavy chain (HC) immunoglobulin variable domain sequence and/or a light chain (LC) immunoglobulin variable domain sequence. In one embodiment, the protein binds to and inhibits plasma kallikrein, e.g., human plasma kallikrein and/or murine kallikrein.

The protein can include one or more of the following characteristics: (a) a human CDR or human framework region; (b) the HC immunoglobulin variable domain sequence comprises one or more (e.g., 1, 2, or 3) CDRs that are at least 85, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, or 100% identical to a CDR of a HC variable domain described herein; (c) the LC immunoglobulin variable domain sequence comprises one or more (e.g., 1, 2, or 3) CDRs that are at least 85, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, or 100% identical to a CDR of a LC variable domain described herein; (d) the LC immunoglobulin variable domain sequence is at least 85, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, or 100% identical to a LC variable domain described herein (e.g., overall or in framework regions or CDRs); (e) the HC immunoglobulin variable domain sequence is at least 85, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, or 100% identical to a HC variable domain described herein (e.g., overall or in framework regions or CDRs); (f) the protein binds an epitope bound by a protein described herein, or competes for binding with a protein described herein; (g) a primate CDR or primate framework region; (h) the HC immunoglobulin variable domain sequence comprises a CDR1 that differs by at least one amino acid but by no more than 2 or 3 amino acids from the CDR1 of a HC variable domain described herein; (i) the HC immunoglobulin variable domain sequence comprises a CDR2 that differs by at least one amino acid but by no more than 2, 3, 4, 5, 6, 7, or 8 amino acids from the CDR2 of a HC variable domain described herein; (j) the HC immunoglobulin variable domain sequence comprises a CDR3 that differs by at least one amino acid but by no more than 2, 3, 4, 5, or 6 amino acids from the CDR3 of a HC variable domain described herein; (k) the LC immunoglobulin variable domain sequence comprises a CDR1 that differs by at least one amino acid but by no more than 2, 3, 4, or 5 amino acids from the CDR1 of a LC variable domain described herein; (l) the LC immunoglobulin variable domain sequence comprises a CDR2 that differs by at least one amino acid but by no more than 2, 3, or 4 amino acids from the CDR2 of a LC variable domain described herein; (m) the LC immunoglobulin variable domain sequence comprises a CDR3 that differs by at least one amino acid but by no more than 2, 3, 4, or 5 amino acids from the CDR3 of a LC variable domain described herein; (n) the LC immunoglobulin variable domain sequence differs by at least one amino acid but by no more than 2, 3, 4, 5, 6, 7, 8, 9, or 10 amino acids from a LC variable domain described herein (e.g., overall or in framework regions or CDRs); and (o) the HC immunoglobulin variable domain sequence differs by at least one amino acid but by no more than 2, 3, 4, 5, 6, 7, 8, 9, or 10 amino acids from a HC variable domain described herein (e.g., overall or in framework regions or CDRs).

The plasma kallikrein binding protein may be an isolated protein (e.g., at least 70, 80, 90, 95, or 99% free of other proteins). In some embodiments, the plasma kallikrein binding protein, or composition thereof, is isolated from antibody cleavage fragments (e.g., cleaved DX-2922) that are inactive or partially active (e.g., bind plasma kallikrein with a Ki, app of 5000 nM or greater) compared to the plasma kallikrein binding protein. For example, the plasma kallikrein binding protein is at least 70% free of such antibody cleavage fragments; in other embodiments the binding protein is at least 80%, at least 90%, at least 95%, at least 99% or even 100% free from antibody cleavage fragments that are inactive or partially active.

The plasma kallikrein binding protein may additionally inhibit plasma kallikrein, e.g., human plasma kallikrein.

In some embodiments, the plasma kallikrein binding protein does not bind prekallikrein (e.g., human prekallikrein and/or murine prekallikrein), but binds to the active form of plasma kallikrein (e.g., human plasma kallikrein and/or murine kallikrein).

In certain embodiments, the protein binds at or near the active site of the catalytic domain of plasma kallikrein, or a fragment thereof, or binds an epitope that overlaps with the active site of plasma kallikrein.

In some aspects, the protein binds the same epitope or competes for binding with a protein described herein.

In some embodiments, the protein competes with or binds the same epitope as M162-A04, M160-G12, M142-H08, X63-G06, X101-A01, X81-B01, X67-D03, X67-G04, X115-B07, X115-D05, X115-E09, X115-H06, X115-A03, X115-D01, X115-F02, X115-G04, M29-D09, M145-D11, M06-D09 and M35-G04.

In some embodiments, the protein binds to (e.g., positions on plasma kallikrein corresponding to) CLIPS peptide C1, C2, C3, C4, C5, C6, or C7, or more than one of these peptides, e.g., the protein binds to C5 and C6. CLIPS peptides C1-C7 are peptides in plasma kallikrein identified by CLIPS epitope mapping (see FIGS. **9** and **10**A-**10**C). C1 corresponds to positions 55-67 of the catalytic domain, C2 to positions 81-94, C3 to positions 101-108, C4 to positions 137-151, C5 to positions 162-178, C6 to positions 186-197, and C7 to positions 214-217 of plasma kallikrein.

In some embodiments, the protein binds to an epitope shown in FIG. **9**.

In some embodiments, the protein binds to one or more amino acids that form the catalytic triad of plasma kallikrein: His434, Asp483, and/or Ser578 (numbering based on the human sequence).

In some embodiments, the protein binds one or more amino acids of: Arg551, Gln553, Tyr555, Thr558, and/or Arg560 (numbering based on the human sequence). In some embodiments, the plasma kallikrein binding protein binds one or more amino acids of: S478, N481, S525, and K526 (numbering based on the human kallikrein sequence).

In some embodiments, the protein binds to one or more amino acids of Ser479, Tyr563, and/or Asp585 (numbering based on the human sequence).

The active site cleft of plasma kallikrein contains three amino acids that form the catalytic triad (His434, Asp483, and Ser578) and result in enzymatic hydrolysis of bound substrate (catalytic triad residues are underlined in FIG. **10**). The peptides selected for the CLIPS epitope mapping analysis were determined to be surface accessible and either form or surround the vicinity of the active site. Peptide C1 contains the active site histidine 434. Peptide C3 contains the active site aspartate 483. Peptide C6 contains the active site serine 578. It is possible for an antibody to bind multiple surface exposed amino acids that are discontinuous in amino acid sequence. For example, by CLIPs analysis, X81-B01 appears to bind the C2, C3, C5 and the C6 peptides.

In some embodiments, the protein binds to an epitope that includes one or more amino acids from CLIPS peptide C1, peptide C2, peptide C3, peptide C4, peptide C5, peptide C6, or peptide C7.

In some embodiments, the protein binds to an epitope that includes amino acids from at least 2 different CLIPS peptides, e.g., from at least two of peptide C1, peptide C2, peptide C3, peptide C4, peptide C5, peptide C6, or peptide C7.

The protein can bind to plasma kallikrein, e.g., human plasma kallikrein, with a binding affinity of at least 10^5, 10^6, 10^7, 10^8, 10^9, 10^{10} and 10^{11} M^{-1}. In one embodiment, the protein binds to human plasma kallikrein with a K_{off} slower than 1×10^{-3}, 5×10^{-4} s^{-1}, or 1×10^{-4} s^{-1}. In one embodiment, the protein binds to human plasma kallikrein with a K_{on} faster than 1×10^2, 1×10^3, or 5×10^3 $M^{-1}s^{-1}$. In one embodiment, the protein binds to plasma kallikrein, but does not bind to tissue kallikrein and/or plasma prekallikrein (e.g., the protein binds to tissue kallikrein and/or plasma prekallikrein less effectively (e.g., 5-, 10-, 50-, 100-, or 1000-fold less or not at all, e.g., as compared to a negative control) than it binds to plasma kallikrein.

In one embodiment, the protein inhibits human plasma kallikrein activity, e.g., with a Ki of less than 10^{-5}, 10^{-6}, 10^{-7}, 10^{-8}, 10^{-9}, and 10^{-10} M. The protein can have, for example, an IC_{50} of less than 100 nM, 10 nM, 1, 0.5, or 0.2 nM. For example, the protein may modulate plasma kallikrein activity, as well as the production of Factor XIIa (e.g., from Factor XII) and/or bradykinin (e.g., from high-molecular-weight kininogen (HMWK)). The protein may inhibit plasma kallikrein activity, and/or the production of Factor XIIa (e.g., from Factor XII) and/or bradykinin (e.g., from high-molecular-weight kininogen (HMWK)). The affinity of the protein for human plasma kallikrein can be characterized by a K_D of less than 100 nm, less than 10 nM, less than 5 nM, less than 1 nM, less than 0.5 nM. In one embodiment, the protein inhibits plasma kallikrein, but does not inhibit tissue kallikrein (e.g., the protein inhibits tissue kallikrein less effectively (e.g., 5-, 10-, 50-, 100-, or 1000-fold less or not at all, e.g., as compared to a negative control) than it inhibits plasma kallikrein.

In some embodiments, the protein has an apparent inhibition constant ($K_{i,app}$) of less than 1000, 500, 100, 5, 1, 0.5 or 0.2 nM.

Plasma kallikrein binding proteins may be antibodies. Plasma kallikrein binding antibodies may have their HC and LC variable domain sequences included in a single polypeptide (e.g., scFv), or on different polypeptides (e.g., IgG or Fab).

In a preferred embodiment, the protein is an antibody (e.g., a human antibody) having the light and heavy chains of antibodies selected from the group consisting of M162-A04, M199-A08, M160-G12, M142-H08 X63-G06, X101-A01, X81-B01, X67-D03, X67-G04, DX-2922, X115-B07, X115-D05, X115-E09, X115-H06, X115-A03, X115-D01, X115-F02, X124-G01, X115-G04, M29-D09, M145-D11, M06-D09 and M35-G04.

In a preferred embodiment, the protein is an antibody (e.g., a human antibody) having the heavy chain of an antibody selected from the group consisting of: M162-A04, M199-A08, M160-G12, M142-H08, X63-G06, X101-A01, X81-B01, X67-D03, X67-G04, X115-B07, X115-D05, X115-E09, X115-H06, X115-A03, X115-D01, X115-F02, X124-G01, X115-G04, M29-D09, M145-D11, M06-D09 and M35-G04.

In a preferred embodiment, the protein is an antibody (e.g., a human antibody) having the light chain of an antibody selected from the group consisting of: M162-A04, M199-A08, M160-G12, M142-H08 X63-G06, X101-A01, X81-B01, X67-D03, X67-G04, X115-B07, X115-D05, X115-E09, X115-H06, X115-A03, X115-D01, X115-F02, X124-G01, X115-G04, M29-D09, M145-D11, M06-D09 and M35-G04.

In a preferred embodiment, the protein is an antibody (e.g., a human antibody) having light and heavy antibody variable regions of an antibody selected from the group consisting of M162-A04, M199-A08, M160-G12, M142-H08, X63-G06, X101-A01, X81-B01, X67-D03, X67-G04, X115-B07, X115-D05, X115-E09, X115-H06, X115-A03, X115-D01, X115-F02, X124-G01, X115-G04, M29-D09, M145-D11, M06-D09 and M35-G04.

In a preferred embodiment, the protein is an antibody (e.g., a human antibody) having a heavy chain antibody variable region of an antibody selected from the group consisting of: M162-A04, M199-A08, M160-G12, M142-H08, X63-G06, X101-A01, X81-B01, X67-D03, X67-G04, X115-B07, X115-D05, X115-E09, X115-H06, X115-A03, X115-D01, X115-F02, X124-G01, X115-G04, M29-D09, M145-D11, M06-D09 and M35-G04.

In a preferred embodiment, the protein is an antibody (e.g., a human antibody) having a light chain antibody variable region of an antibody selected from the group consisting of: M162-A04, M199-A08, M160-G12, M142-H08, X63-G06, X101-A01, X81-B01, X67-D03, X67-G04, X115-B07, X115-D05, X115-E09, X115-H06, X115-A03, X115-D01, X115-F02, X124-G01, X115-G04, M29-D09, M145-D11, M06-D09 and M35-G04.

In a preferred embodiment, the protein is an antibody (e.g., a human antibody) having one or more (e.g., 1, 2, or 3) heavy chain CDRs selected from the corresponding CDRs of the group of heavy chains consisting of M162-A04, M199-A08, M160-G12, M142-H08, X63-G06, X101-A01, X81-B01, X67-D03, X67-G04, X115-B07, X115-D05, X115-E09, X115-H06, X115-A03, X115-D01, X115-F02, X124-G01, X115-G04, M29-D09, M145-D11, M06-D09 and M35-G04.

In a preferred embodiment, the protein is an antibody (e.g., a human antibody) having one or more (e.g., 1, 2, or 3) light chain CDRs selected from the corresponding CDRs of the group of light chains consisting of M162-A04, M199-A08, M160-G12, M142-H08, X63-G06, X101-A01, X81-B01, X67-D03, X67-G04, X115-B07, X115-D05, X115-E09, X115-H06, X115-A03, X115-D01, X115-F02, X124-G01, X115-G04, M29-D09, M145-D11, M06-D09 and M35-G04.

In a preferred embodiment, the protein is an antibody (e.g., a human antibody) having one or more (e.g., 1, 2, or 3) heavy chain CDRs and one or more (e.g., 1, 2, or 3) light chain CDRs selected from the corresponding CDRs of the group of light chains consisting of M162-A04, M199-A08, M160-G12, M142-H08, X63-G06, X101-A01, X81-B01, X67-D03, X67-G04, X115-B07, X115-D05, X115-E09, X115-H06, X115-A03, X115-D01, X115-F02, X124-G01, X115-G04, M29-D09, M145-D11, M06-D09 and M35-G04.

In one embodiment, the HC and LC variable domain sequences are components of the same polypeptide chain. In another, the HC and LC variable domain sequences are components of different polypeptide chains. For example, the protein is an IgG, e.g., IgG1, IgG2, IgG3, or IgG4. The protein can be a soluble Fab. In other implementations the protein includes a Fab2', scFv, minibody, scFv::Fc fusion, Fab::HSA fusion, HSA::Fab fusion, Fab::HSA::Fab fusion, or other molecule that comprises the antigen combining site of one of the binding proteins herein. The VH and VL regions of these Fabs can be provided as IgG, Fab, Fab2, Fab2', scFv, PEGylated Fab, PEGylated scFv, PEGylated Fab2, VH::CH1::HSA+LC, HSA::VH::CH1+LC, LC::HSA+VH::CH1, HSA::LC+VH::CH1, or other appropriate construction.

In one embodiment, the protein is a human or humanized antibody or is non-immunogenic in a human. For example, the protein includes one or more human antibody framework regions, e.g., all human framework regions, or framework regions at least 85, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99% identical to human framework regions. In one embodiment, the protein includes a human Fc domain, or an Fc domain that is at least 95, 96, 97, 98, or 99% identical to a human Fc domain.

In one embodiment, the protein is a primate or primatized antibody or is non-immunogenic in a human. For example, the protein includes one or more primate antibody framework regions, e.g., all primate framework regions, or framework regions at least 85, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99% identical to primate framework regions. In one embodiment, the protein includes a primate Fc domain, or an Fc domain that is at least 95, 96, 97, 98, or 99% identical to a primate Fc domain. "Primate" includes humans (*Homo sapiens*), chimpanzees (*Pan troglodytes* and *Pan paniscus* (bonobos)), gorillas (*Gorilla gorilla*), gibons, monkeys, lemurs, aye-ayes (*Daubentonia madagascariensis*), and tarsiers.

In some embodiments, the affinity of the primate antibody for human plasma kallikrein is characterized by a K_D of less than 1000, 500, 100, 10, 5, 1, 0.5 nM, e.g., less than 10 nM, less than 1 nM, or less than 0.5 nM.

In certain embodiments, the protein includes no sequences from mice or rabbits (e.g., is not a murine or rabbit antibody).

In some aspects, the disclosure provides the use of proteins (e.g., binding proteins, e.g., antibodies) (e.g., the proteins described herein) that bind to plasma kallikrein (e.g., human plasma kallikrein) and include at least one immunoglobin variable region in methods for treating (or preventing) a plasma kallikrein associated disorder or condition. For example, the plasma kallikrein binding protein includes a heavy chain (HC) immunoglobulin variable domain sequence and a light chain (LC) immunoglobulin variable domain sequence. A number of exemplary plasma kallikrein binding proteins are described herein.

The plasma kallikrein binding protein may be an isolated protein (e.g., at least 70, 80, 90, 95, or 99% free of other proteins).

The plasma kallikrein binding protein may additionally inhibit plasma kallikrein, e.g., human plasma kallikrein and/or murine plasma kallikrein. In some embodiments, it may be preferred to have an plasma kallikrein binding protein bind to both human and murine plasma kallikrein, as these antibodies can be tested for efficacy in a mouse model.

Plasma Kallikrein

Exemplary plasma kallikrein sequences against which plasma kallikrein binding proteins may be developed can include human, mouse, or rat plasma kallikrein amino acid sequences, a sequence that is 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to one of these sequences, or a fragment thereof, e.g., of a sequence provided below.

The sequence of human plasma kallikrein that was used in selections and subsequent screening is shown below (accession number NP_000883.2). The human plasma kallikrein (86 kDa) that was used was purified from human plasma and activated with factor XIIa by a commercial vendor. Factor XIIa activates prekallikrein by cleaving the polypeptide sequence at a single site (between Arg371-Ile372, cleavage site marked by "/" in the sequence below) to generate active plasma kallikrein, which then consists of two disulfide linked polypeptides; a heavy chain of approximately 52 kDa and a catalytic domain of approximately 34 kDa [Colman and Schmaier, (1997) "Contact System: A Vascular Biology Modulator With Anticoagulant, Profibrinolytic, Antiadhesive, and Proinflammatory Attributes" Blood, 90, 3819-3843]

```
GCLTQLYENAFFRGGDVASMYTPNAQYCQMRCTFHPRCLLFSFLPASSIND

MEKRFGCFLKDSVTGTLPKVHRTGAVSGHSLKQCGHQISACHRDIYKGVDM

RGVNFNVSKVSSVEECQKRCTSNIRCQFFSYATQTFHKAEYRNNCLLKYSP
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GGTPTAIKVLSNVESGFSLKPCALSEIGCHMNIFQHLAFSDVDVARVLTPD

AFVCRTICTYHPNCLFFTFYTNVWKIESQRNVCLLKTSESGTPSSSTPQEN

TISGYSLLTCKRTLPEPCHSKIYPGVDFGGEELNVTFVKGVNVCQETCTKM

IRCQFFTYSLLPEDCKEEKCKCFLRLSMDGSPTRIAYGTQGSSGYSLRLCN

TGDNSVCTTKTSTR/IVGGTNSSWGEWPWQVSLQVKLTAQRHLCGGSLIGH

QWVLTAAHCFDGLPLQDVWRIYSGILNLSDITKDTPFSQIKEIIIHQNYKV

SEGNHDIALIKLQAPLNYTEFQKPICLPSKGDTSTIYTNCWVTGWGFSKEK

GEIQNILQKVNIPLVTNEECQKRYQDYKITQRMVCAGYKEGGKDACKGDSG

GPLVCKHNGMWRLVGITSWGEGCARREQPGVYTKVAEYMDWILEKTQSSDG

KAQMQSPA
```

The human, mouse, and rat prekallikrein amino acid sequences, and the mRNA sequences encoding the same, are illustrated below. The sequences of prekallikrein are the same as plasma kallikrein, except that active plasma kallikrein (pkal) has the single polypeptide chain cleaved at a single position (indicated by the "/") to generate two chains. The sequences provided below are full sequences that include signal sequences. On secretion from the expressing cell, it is expected that the signal sequences are removed.

Human Plasma Kallikrein (Accession: NP_000883.2)

```
>gi|78191798|ref|NP_000883.2| plasma kallikrein B1
precursor[Homo sapiens]
MILFKQATYFISLFATVSCGCLTQLYENAFFRGGDVASMYTPNAQYCQMR

CTFHPRCLLFSFLPASSINDMEKRFGCFLKDSVTGTLPKVHRTGAVSGHS

LKQCGHQISACHRDIYKGVDMRGVNFNVSKVSSVEECQKRCTSNIRCQFF

SYATQTFHKAEYRNNCLLKYSPGGTPTAIKVLSNVESGFSLKPCALSEIG

CHMNIFQHLAFSDVDVARVLTPDAFVCRTICTYHPNCLFFTFYTNVWKIE

SQRNVCLLKTSESGTPSSSTPQENTISGYSLLTCKRTLPEPCHSKIYPGV

DFGGEELNVTFVKGVNVCQETCTKMIRCQFFTYSLLPEDCKEEKCKCFLR

LSMDGSPTRIAYGTQGSSGYSLRLCNTGDNSVCTTKTSTRIVGGTNSSWG

EWPWQVSLQVKLTAQRHLCGGSLIGHQWVLTAAHCFDGLPLQDVWRIYSG

ILNLSDITKDTPFSQIKEIIIHQNYKVSEGNHDIALIKLQAPLNYTEFQK

PICLPSKGDTSTIYTNCWVTGWGFSKEKGEIQNILQKVNIPLVTNEECQK

RYQDYKITQRMVCAGYKEGGKDACKGDSGGPLVCKHNGMWRLVGITSWGE

GCARREQPGVYTKVAEYMDWILEKTQSSDGKAQMQSPA
```

Human Plasma Kallikrein mRNA (Accession: NM_000892)

```
>gi|78191797|ref|NM_000892.3| Homo sapiens
kallikrein B, plasma(Fletcher factor) 1 (KLKB1),
mRNA
AGAACAGCTTGAAGACCGTTCATTTTTAAGTGACAAGAGACTCACCTCCAA

GAAGCAATTGTGTTTTCAGAATGATTTTATTCAAGCAAGCAACTTATTTCA

TTTCCTTGTTTGCTACAGTTTCCTGTGGATGTCTGACTCAACTCTATGAAA

ACGCCTTCTTCAGAGGTGGGGATGTAGCTTCCATGTACACCCCAAATGCCC

AATACTGCCAGATGAGGTGCACATTCCACCCAAGGTGTTTGCTATTCAGTT
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TTCTTCCAGCAAGTTCAATCAATGACATGGAGAAAAGGTTTGGTTGCTTCT

TGAAAGATAGTGTTACAGGAACCCTGCCAAAAGTACATCGAACAGGTGCAG

TTTCTGGACATTCCTTGAAGCAATGTGGTCATCAAATAAGTGCTTGCCATC

GAGACATTTATAAAGGAGTTGATATGAGAGGAGTCAATTTTAATGTGTCTA

AGGTTAGCAGTGTTGAAGAATGCCAAAAAAGGTGCACCAGTAACATTCGCT

GCCAGTTTTTTCATATGCCACGCAAACATTTCACAAGGCAGAGTACCGGA

ACAATTGCCTATTAAAGTACAGTCCCGGAGGAACACCTACCGCTATAAAGG

TGCTGAGTAACGTGGAATCTGGATTCTCACTGAAGCCCTGTGCCCTTTCAG

AAATTGGTTGCCACATGAACATCTTCCAGCATCTTGCGTTCTCAGATGTGG

ATGTTGCCAGGGTTCTCACTCCAGATGCTTTTGTGTGTCGGACCATCTGCA

CCTATCACCCCAACTGCCTCTTCTTTACATTCTATACAAATGTATGGAAAA

TCGAGTCACAAAGAAATGTTTGTCTTCTTAAAACATCTGAAAGTGGCACAC

CAAGTTCCTCTACTCCTCAAGAAAACACCATATCTGGATATAGCCTTTTAA

CCTGCAAAAGAACTTTACCTGAACCCTGCCATTCTAAAATTTACCCGGGAG

TTGACTTTGGAGGAGAAGAATTGAATGTGACTTTTGTTAAAGGAGTGAATG

TTTGCCAAGAGACTTGCACAAAGATGATTCGCTGTCAGTTTTTCACTTATT

CTTTACTCCCAGAAGACTGTAAGGAAGAGAAGTGTAAGTGTTTCTTAAGAT

TATCTATGGATGGTTCTCCAACTAGGATTGCGTATGGGACACAAGGGAGCT

CTGGTTACTCTTTGAGATTGTGTAACACTGGGGACAACTCTGTCTGCACAA

CAAAAACAAGCACACGCATTGTTGGAGGAACAAACTCTTCTTGGGGAGAGT

GGCCCTGGCAGGTGAGCCTGCAGGTGAAGCTGACAGCTCAGAGGCACCTGT

GTGGAGGGTCACTCATAGGACACCAGTGGGTCCTCACTGCTGCCCACTGCT

TTGATGGGCTTCCCCTGCAGGATGTTTGGCGCATCTATAGTGGCATTTTAA

ATCTGTCAGACATTACAAAAGATACACCTTTCTCACAAATAAAAGAGATTA

TTATTCACCAAAACTATAAAGTCTCAGAAGGGAATCATGATATCGCCTTGA

TAAAACTCCAGGCTCCTTTGAATTACACTGAATTCCAAAAACCAATATGCC

TACCTTCCAAAGGTGACACAAGCACAATTTATACCAACTGTTGGGTAACCG

GATGGGGCTTCTCGAAGGAGAAAGGTGAAATCCAAAATATTCTACAAAAGG

TAAATATTCCTTTGGTAACAAATGAAGAATGCCAGAAAAGATATCAAGATT

ATAAAATAACCCAACGGATGGTCTGTGCTGGCTATAAAGAAGGGGGAAAAG

ATGCTTGTAAGGGAGATTCAGGTGGTCCCTTAGTTTGCAAACACAATGGAA

TGTGGCGTTTGGTGGGCATCACCAGCTGGGGTGAAGGCTGTGCCCGCAGGG

AGCAACCTGGTGTCTACACCAAAGTCGCTGAGTACATGGACTGGATTTTAG

AGAAAACACAGAGCAGTGATGGAAAAGCTCAGATGCAGTCACCAGCATGAG

AAGCAGTCCAGAGTCTAGGCAATTTTTACAACCTGAGTTCAAGTCAAATTC

TGAGCCTGGGGGGTCCTCATCTGCAAAGCATGGAGAGTGGCATCTTCTTTG

CATCCTAAGGACGAAAAACACAGTGCACTCAGAGCTGCTGAGGACAATGTC

TGGCTGAAGCCCGCTTTCAGCACGCCGTAACCAGGGGCTGACAATGCGAGG

TCGCAACTGAGATCTCCATGACTGTGTGTTGTGAAATAAAATGGTGAAAGA

TCAAAAAA

Mouse Plasma Kallikrein (Accession: NP_032481.1)

>gi|6680584|ref|NP_032481.1| kallikrein B, plasma 1 [*Mus musculus*]

MILFNRVGYFVSLFATVSCGCMTQLYKNTFFRGGDLAAIYTPDAQYCQKMC

TFHPRCLLFSFLAVTPPKETNKRFGCFMKESITGTLPRIHRTGAISGHSLK

QCGHQISACHRDIYKGLDMRGSNFNISKTDNIEECQKLCTNNFHCQFFTYA

TSAFYRPEYRKKCLLKHSASGTPTSIKSADNLVSGFSLKSCALSEIGCPMD

IFQHSAFADLNVSQVITPDAFVCRTICTFHPNCLFFTFYTNEWETESQRNV

CFLKTSKSGRPSPPIPQENAISGYSLLTCRKTRPEPCHSKIYSGVDFEGEE

LNVTFVQGADVCQETCTKTIRCQFFIYSLLPQDCKEEGCKCSLRLSTDGSP

TRITYGMQGSSGYSLRLCKLVDSPDCTTKINARIVGGTNASLGEWPWQVSL

QVKLVSQTHLCGGSIIGRQWVLTAAHCFDGIPYPDVWRIYGGILSLSEITK

ETPSSRIKELIIHQEYKVSEGNYDIALIKLQTPLNYTEFQKPICLPSKADT

NTIYTNCWVTGWGYTKEQGETQNILQKATIPLVPNEECQKKYRDYVINKQM

ICAGYKEGGTDACKGDSGGPLVCKHSGRWQLVGITSWGEGCGRKDQPGVYT

KVSEYMDWILEKTQSSDVRALETSSA

Mouse Plasma Kallikrein mRNA (Accession: NM_008455.2)

>gi|236465804|ref|NM_008455.2| *Mus musculus* kallikrein B, plasma 1(Klkb1), mRNA

AGACCGCCCTCGGTGCCATATTCAGAGGGCTTGAAGACCATCTTCATGTG

AAGACTCCCTCTCCTCCAGAACCACAACGTGACCATCCTTCCAGGATGAT

TTTATTCAACCGAGTGGGTTATTTTGTTTCCTTGTTTGCTACCGTCTCCT

GTGGGTGTATGACTCAACTGTATAAAAATACCTTCTTCAGAGGTGGGGAT

CTAGCTGCCATCTACACCCCAGATGCCCAGTACTGTCAGAAGATGTGCAC

TTTTCACCCCAGGTGCCTGCTGTTCAGCTTTCTCGCCGTGACTCCACCCA

AAGAGACAAATAAACGGTTTGGTTGCTTCATGAAAGAGAGCATTACAGGG

ACTTTGCCAAGAATACACCGGACAGGGGCCATTTCTGGTCATTCTTTAAA

GCAGTGTGGCCATCAAATAAGTGCTTGCCACCGAGACATATACAAAGGAC

TTGATATGAGAGGGTCCAACTTTAATATCTCTAAGACCGACAATATTGAA

GAATGCCAGAAACTGTGCACAAATAATTTTCACTGCCAATTTTTCACATA

TGCTACAAGTGCATTTTACAGACCAGAGTACCGGAAGAAGTGCCTGCTGA

AGCACAGTGCAAGCGGAACACCCACCAGCATAAAGTCAGCGGACAACCTG

GTGTCTGGATTCTCACTGAAGTCCTGTGCGCTTTCGGAGATAGGTTGCCC

CATGGATATTTTCCAGCACTCTGCCTTTGCAGACCTGAATGTAAGCCAGG

TCATCACCCCCGATGCCTTTGTGTGTCGCACCATCTGCACCTTCCATCCC

AACTGCCTTTTCTTCACGTTCTACACGAATGAATGGGAGACAGAATCACA

GAGAAATGTTTGTTTTCTTAAGACGTCTAAAAGTGGAAGACCAAGTCCCC

CTATTCCTCAAGAAAACGCTATATCTGGATATAGTCTCAAACTCGCCCTG

AACCCTGCCATTCCAAACTCACCTGCAGAAATTTACTCTGGAGTTGACTT

TGAAGGGGAAGAACTGAATGTGACCTTCGTGCAAGGAGCAGATGTCTGCC

AAGAGACTTGTACAAAGACAATCCGCTGCCAGTTTTTTATTTACTCCTTA

-continued

CTCCCCCAAGACTGCAAGGAGGAGGGGTGTAAATGTTCCTTAAGGTTATC

CACAGATGGCTCCCCAACTAGGATCACCTATGGCATGCAGGGGAGCTCCG

GTTATTCTCTGAGATTGTGTAAACTTGTGGACAGCCCTGACTGTACAACA

AAAATAAATGCACGTATTGTGGGAGGAACAAACGCTTCTTTAGGGGAGTG

GCCATGGCAGGTCAGCCTGCAAGTGAAGCTGGTATCTCAGACCCATTTGT

GTGGAGGGTCCATCATTGGTCGCCAATGGGTACTGACAGCTGCCCATTGC

TTTGATGGAATTCCCTATCCAGATGTGTGGCGTATATATGGCGGAATTCT

TAGTCTGTCCGAGATTACGAAAGAAACGCCTTCCTCGAGAATAAAGGAGC

TTATTATTCATCAGGAATACAAAGTCTCAGAAGGCAATTATGATATTGCC

TTAATAAAGCTTCAGACGCCCCTGAATTATACTGAATTCCAAAAACCAAT

ATGCCTGCCTTCCAAAGCTGACACAAATACAATTTATACCAACTGTTGGG

TGACTGGATGGGGCTACACGAAGGAACAAGGTGAAACGCAAAATATTCTA

CAAAAGGCTACTATTCCTTTGGTACCAAATGAAGAATGCCAGAAAAAATA

CAGAGATTATGTTATAAACAAGCAGATGATCTGTGCTGGCTACAAAGAAG

GCGGAACAGACGCTTGTAAGGGAGATTCCGGTGGCCCCTTAGTCTGTAAA

CACAGTGGACGGTGGCAGTTGGTGGGTATCACCAGCTGGGGTGAAGGCTG

CGCCCGCAAGGACCAACCAGGAGTCTACACCAAAGTTTCTGAGTACATGG

ACTGGATATTGGAGAAGACACAGAGCAGTGATGTAAGAGCTCTGGAGACA

TCTTCAGCCTGAGGAGGCTGGGTACCAAGGAGGAAGAACCCAGCTGGCTT

TACCACCTGCCCTCAAGGCAAACTAGAGCTCCAGGATTCTCGGCTGTAAA

ATGTTGATAATGGTGTCTACCTCACATCCGTATCATTGGATTGAAAATTC

AAGTGTAGATATAGTTGCTGAAGACAGCGTTTTGCTCAAGTGTGTTTCCT

GCCTTGAGTCACAGGAGCTCCAATGGGAGCATTACAAAGATCACCAAGCT

TGTTAGGAAAGAGAATGATCAAAGGGTTTTATTAGGTAATGAAATGTCTA

GATGTGATGCAATTGAAAAAAAGACCCCAGATTCTAGCACAGTCCTTGGG

ACCATTCTCATGTAACTGTTGACTCTGGACCTCAGCAGATCTCAGAGTTA

CCTGTCCACTTCTGACATTTGTTTATTAGAGCCTGATGCTATTCTTTCAA

GTGGAGCAAAAAAAAAAAAAAA

Rat Plasma Kallikrein (Accession: NP_036857.2)

>gi|162138905|ref|NP_036857.2| kallikrein B, plasma 1 [*Rattus norvegicus*]

MILFKQVGYFVSLFATVSCGCLSQLYANTFFRGGDLAAIYTPDAQHCQKM

CTFHPRCLLFSFLAVSPTKETDKRFGCFMKESITGTLPRIHRTGAISGHS

LKQCGHQLSACHQDIYEGLDMRGSNFNISKTDSIEECQKLCTNNIHCQFF

TYATKAFHRPEYRKSCLLKRSSSGTPTSIKPVDNLVSGFSLKSCALSEIG

CPMDIFQHFAFADLNVSHVVTPDAFVCRTVCTFHPNCLFFTFYTNEWETE

SQRNVCFLKTSKSGRPSPPIIQENAVSGYSLFTCRKARPEPCHFKIYSGV

AFEGEELNATFVQGADACQETCTKTIRCQFFTYSLLPQDCKAEGCKCSLR

LSTDGSPTRITYEAQGSSGYSLRLCKVVESSDCTTKINARIVGGTNSSLG

HEWPWQVSLQVKLVSQNMCGGSIIGRQWILTAAHCFDGIPYPDVWRIYGG

-continued

ILNLSEITNKTPFSSIKELIIHQKYKMSEGSYDIALIKLQTPLNYTEFQK

PICLPSKADTNTIYTNCWVTGWGYTKERGETQNILQKATIPLVPNEECQK

KYRDYVITKQMICAGYKEGGIDACKGDSGGPLVCKHSGRWQLVGITSWGE

GCARKEQPGVYTKVAEYIDWILEKIQSSKERALETSPA

Rat Plasma Kallikrein mRNA (Accession: NM_012725)

>gi|162138904|ref|NM_012725.21 *Rattus norvegicus* kallikrein B, plasma 1(Klkb1), mRNA

TGAAGACTAGCTTCATGTGAAGACTCCTTCTCCTCCAGCAGCACAAAGCA

ACCATCCTTCCAGGATGATTTTATTCAAACAAGTGGGTTATTTTGTTTCC

TTGTTCGCTACAGTTTCCTGTGGGTGTCTGTCACAACTGTATGCAAATAC

CTTCTTCAGAGGTGGGGATCTGGCTGCCATCTACACCCCGGATGCCCAGC

ACTGTCAGAAGATGTGCACGTTTCACCCCAGGTGCCTGCTCTTCAGCTTC

CTTGCCGTGAGTCCAACCAAGGAGACAGATAAAAGGTTTGGGTGCTTCAT

GAAAGAGAGCATTACAGGGACTTTGCCAAGAATACACCGGACAGGGGCCA

TTTCTGGTCATTCTTTAAAACAGTGTGGCCATCAATTAAGTGCTTGCCAC

CAAGACATATACGAAGGACTGGATATGAGAGGGTCCAACTTTAATATATC

TAAGACCGACAGTATTGAAGAATGCCAGAAACTGTGCACAAATAATATTC

ACTGCCAATTTTTCACATATGCTACAAAAGCATTTCACAGACCAGAGTAC

AGGAAGAGTTGCCTGCTGAAGCGCAGTTCAAGTGGAACGCCCACCAGTAT

AAAGCCAGTGGACAACCTGGTGTCTGGATTCTCACTGAAGTCCTGTGCTC

TCTCAGAGATCGGTTGCCCCATGGATATTTTCCAGCACTTTGCCTTTGCA

GACCTGAATGTAAGCCATGTCGTCACCCCCGATGCCTTCGTGTGTCGCAC

CGTTTGCACCTTCCATCCCAACTGCCTCTTCTTCACATTCTACACGAATG

AGTGGGAGACGGAATCACAGAGGAATGTTTGTTTTCTTAAGACATCTAAA

AGTGGAAGACCAAGTCCCCCTATTATTCAAGAAAATGCTGTATCTGGATA

CAGTCTCTTCACCTGCAGAAAAGCTCGCCCTGAACCCTGCCATTTCAAGA

TTTACTCTGGAGTTGCCTTCGAAGGGGAAGAACTGAACGCGACCTTCGTG

CAGGGAGCAGATGCGTGCCAAGAGACTTGTACAAAGACCATCCGCTGTCA

GTTTTTTACTTACTCATTGCTTCCCCAAGACTGCAAGGCAGAGGGGTGTA

AATGTTCCTTAAGGTTATCCACGGATGGCTCTCCAACTAGGATCACCTAT

GAGGCACAGGGGAGCTCTGGTTATTCTCTGAGACTGTGTAAAGTTGTGGA

GAGCTCTGACTGTACGACAAAAATAAATGCACGTATTGTGGGAGGAACAA

ACTCTTCTTTAGGAGAGTGGCCATGGCAGGTCAGCCTGCAAGTAAAGTTG

GTTTCTCAGAATCATATGTGTGGAGGGTCCATCATTGGACGCCAATGGAT

ACTGACGGCTGCCCATTGCTTTGATGGGATTCCCTATCCAGACGTGTGGC

GTATATATGGCGGGATTCTTAATCTGTCAGAGATTACAAACAAAACGCCT

TTCTCAAGTATAAAGGAGCTTATTATTCATCAGAAATACAAAATGTCAGA

AGGCAGTTACGATATTGCCTTAATAAAGCTTCAGACACCGTTGAATTATA

CTGAATTCCAAAAACCAATATGCCTGCCTTCCAAAGCTGACACAAATACA

ATTTATACCAACTGCTGGGTGACTGGATGGGGCTACACAAAGGAACGAGG

TGAGACCCAAAATATTCTACAAAAGGCAACTATTCCCTTGGTACCAAATG

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AAGAATGCCAGAAAAAATATAGAGATTATGTTATAACCAAGCAGATGATC

TGTGCTGGCTACAAAGAAGGTGGAATAGATGCTTGTAAGGGAGATTCCGG

TGGCCCCTTAGTTTGCAAACATAGTGGAAGGTGGCAGTTGGTGGGTATCA

CCAGCTGGGGCGAAGGCTGTGCCCGCAAGGAGCAACCAGGAGTCTACACC

AAAGTTGCTGAGTACATTGACTGGATATTGGAGAAGATACAGAGCAGCAA

GGAAAGAGCTCTGGAGACATCTCCAGCATGAGGAGGCTGGGTACTGATGG

GGAAGAGCCCAGCTGGCACCAGCTTTACCACCTGCCCTCAAGTCCTACTA

GAGCTCCAGAGTTCTCTTCTGCAAAATGTCGATAGTGGTGTCTACCTCGC

ATCCTTACCATAGGATTAAAAGTCCAAATGTAGACACAGTTGCTAAAGAC

AGCGCCATGCTCAAGCGTGCTTCCTGCCTTGAGCAACAGGAACGCCAATG

AGAACTATCCAAAGATTACCAAGCCTGTTTGGAAATAAAATGGTCAAAGG

ATTTTTATTAGGTAGTGAAATTAGGTAGTTGTCCTTGGAACCATTCTCAT

GTAACTGTTGACTCTGGACCTCAGCAGATCACAGTTACCTTCTGTCCACT

TCTGACATTTGTGTACTGGAACCTGATGCTGTTCTTCCACTTGGAGCAAA

GAACTGAGAAACCTGGTTCTATCCATTGGGAAAAAGAGATCTTTGTAACA

TTTCCTTTACAATAAAAAGATGTTCTACTTGGACTTGAAAAAAAAAAAAA

AAAAAAAAAAAAA
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Display Libraries

A display library is a collection of entities; each entity includes an accessible polypeptide component and a recoverable component that encodes or identifies the polypeptide component. The polypeptide component is varied so that different amino acid sequences are represented. The polypeptide component can be of any length, e.g. from three amino acids to over 300 amino acids. A display library entity can include more than one polypeptide component, for example, the two polypeptide chains of a sFab. In one exemplary implementation, a display library can be used to identify proteins that bind to plasma kallikrein. In a selection, the polypeptide component of each member of the library is probed with plasma kallikrein (or fragment thereof) and if the polypeptide component binds to the plasma kallikrein, the display library member is identified, typically by retention on a support.

Retained display library members are recovered from the support and analyzed. The analysis can include amplification and a subsequent selection under similar or dissimilar conditions. For example, positive and negative selections can be alternated. The analysis can also include determining the amino acid sequence of the polypeptide component and purification of the polypeptide component for detailed characterization.

A variety of formats can be used for display libraries. Examples include the following.

Phage Display:

The protein component is typically covalently linked to a bacteriophage coat protein. The linkage results from translation of a nucleic acid encoding the protein component fused to the coat protein. The linkage can include a flexible peptide linker, a protease site, or an amino acid incorporated as a result of suppression of a stop codon. Phage display is described, for example, in U.S. Pat. No. 5,223,409; Smith (1985) *Science* 228:1315-1317; WO 92/18619; WO 91/17271; WO 92/20791; WO 92/15679; WO 93/01288; WO 92/01047; WO 92/09690; WO 90/02809; de Haard et al. (1999) J. Biol. Chem 274:18218-30; Hoogenboom et al. (1998) Immunotechnology 4:1-20; Hoogenboom et al. (2000) Immunol Today 2:371-8 and Hoet et al. (2005) Nat Biotechnol. 23(3)344-8. Bacteriophage displaying the protein component can be grown and harvested using standard phage preparatory methods, e.g. PEG precipitation from growth media. After selection of individual display phages, the nucleic acid encoding the selected protein components can be isolated from cells infected with the selected phages or from the phage themselves, after amplification. Individual colonies or plaques can be picked, the nucleic acid isolated and sequenced.

Other Display Formats.

Other display formats include cell based display (see, e.g., WO 03/029456), protein-nucleic acid fusions (see, e.g., U.S. Pat. No. 6,207,446), ribosome display (See, e.g., Mattheakis et al. (1994) Proc. Natl. Acad. Sci. USA 91:9022 and Hanes et al. (2000) Nat Biotechnol. 18:1287-92; Hanes et al. (2000) Methods Enzymol. 328:404-30; and Schaffitzel et al. (1999) J Immunol Methods. 231(1-2):119-35), and *E. coli* periplasmic display (J Immunol Methods. 2005 Nov. 22; PMID: 16337958).

Scaffolds.

Scaffolds useful for display include: antibodies (e.g., Fab fragments, single chain Fv molecules (scFv), single domain antibodies, camelid antibodies, and camelized antibodies); T-cell receptors; MHC proteins; extracellular domains (e.g., fibronectin Type III repeats, EGF repeats); protease inhibitors (e.g., Kunitz domains, ecotin, BPTI, and so forth); TPR repeats; trifoil structures; zinc finger domains; DNA-binding proteins; particularly monomeric DNA binding proteins; RNA binding proteins; enzymes, e.g., proteases (particularly inactivated proteases), RNase; chaperones, e.g., thioredoxin and heat shock proteins; intracellular signaling domains (such as SH2 and SH3 domains); linear and constrained peptides; and linear peptide substrates. Display libraries can include synthetic and/or natural diversity. See, e.g., U.S. 2004-0005709.

Display technology can also be used to obtain binding proteins (e.g., antibodies) that bind particular epitopes of a target. This can be done, for example, by using competing non-target molecules that lack the particular epitope or are mutated within the epitope, e.g., with alanine. Such non-target molecules can be used in a negative selection procedure as described below, as competing molecules when binding a display library to the target, or as a pre-elution agent, e.g., to capture in a wash solution dissociating display library members that are not specific to the target.

Iterative Selection.

In one preferred embodiment, display library technology is used in an iterative mode. A first display library is used to identify one or more binding proteins for a target. These identified binding proteins are then varied using a mutagenesis method to form a second display library. Higher affinity binding proteins are then selected from the second library, e.g., by using higher stringency or more competitive binding and washing conditions.

In some implementations, the mutagenesis is targeted to regions at the binding interface. If, for example, the identified binding proteins are antibodies, then mutagenesis can be directed to the CDR regions of the heavy or light chains as described herein. Further, mutagenesis can be directed to framework regions near or adjacent to the CDRs. In the case of antibodies, mutagenesis can also be limited to one or a few of the CDRs, e.g., to make precise step-wise improvements. Exemplary mutagenesis techniques include: error-prone PCR, recombination, DNA shuffling, site-directed mutagenesis and cassette mutagenesis.

In one example of iterative selection, the methods described herein are used to first identify a protein from a display library that binds plasma kallikrein, with at least a minimal binding specificity for a target or a minimal activity, e.g., an equilibrium dissociation constant for binding of less than 0.5 nM, 1 nM, 10 nM, or 100 nM. The nucleic acid sequences encoding the initial identified proteins are used as a template nucleic acid for the introduction of variations, e.g., to identify a second protein that has enhanced properties (e.g., binding affinity, kinetics, or stability) relative to the initial protein.

Off-Rate Selection.

Since a slow dissociation rate can be predictive of high affinity, particularly with respect to interactions between polypeptides and their targets, the methods described herein can be used to isolate binding proteins with a desired (e.g., reduced) kinetic dissociation rate for a binding interaction to a target.

To select for slow dissociating binding proteins from a display library, the library is contacted to an immobilized target. The immobilized target is then washed with a first solution that removes non-specifically or weakly bound biomolecules. Then the bound binding proteins are eluted with a second solution that includes a saturating amount of free target or a target specific high-affinity competing monoclonal antibody, i.e., replicates of the target that are not attached to the particle. The free target binds to biomolecules that dissociate from the target. Rebinding is effectively prevented by the saturating amount of free target relative to the much lower concentration of immobilized target.

The second solution can have solution conditions that are substantially physiological or that are stringent. Typically, the solution conditions of the second solution are identical to the solution conditions of the first solution. Fractions of the second solution are collected in temporal order to distinguish early from late fractions. Later fractions include biomolecules that dissociate at a slower rate from the target than biomolecules in the early fractions.

Further, it is also possible to recover display library members that remain bound to the target even after extended incubation. These can either be dissociated using chaotropic conditions or can be amplified while attached to the target. For example, phage bound to the target can be contacted to bacterial cells.

Selecting or Screening for Specificity.

The display library screening methods described herein can include a selection or screening process that discards display library members that bind to a non-target molecule. Examples of non-target molecules include streptavidin on magnetic beads, blocking agents such as bovine serum albumin, non-fat bovine milk, soy protein, any capturing or target immobilizing monoclonal antibody, or non-transfected cells which do not express the target.

In one implementation, a so-called "negative selection" step is used to discriminate between the target and related non-target molecule and a related, but distinct non-target molecule. The display library or a pool thereof is contacted to the non-target molecule. Members of the sample that do not bind the non-target are collected and used in subsequent selections for binding to the target molecule or even for subsequent negative selections. The negative selection step can be prior to or after selecting library members that bind to the target molecule.

In another implementation, a screening step is used. After display library members are isolated for binding to the target molecule, each isolated library member is tested for its ability to bind to a non-target molecule (e.g., a non-target listed above). For example, a high-throughput ELISA screen can be used to obtain this data. The ELISA screen can also be used to obtain quantitative data for binding of each library member to the target as well as for cross species reactivity to related targets or subunits of the target (e.g., plasma kallikrein) and also under different condition such as pH 6 or pH 7.5. The non-target and target binding data are compared (e.g., using a computer and software) to identify library members that specifically bind to the target.

Other Exemplary Expression Libraries

Other types of collections of proteins (e.g., expression libraries) can be used to identify proteins with a particular property (e.g., ability to bind plasma kallikrein), including, e.g., protein arrays of antibodies (see, e.g., De Wildt et al. (2000) Nat. Biotechnol. 18:989-994), lambda gt11 libraries, two-hybrid libraries and so forth.

Exemplary Libraries

It is possible to immunize a non-human primate and recover primate antibody genes that can be displayed on phage (see below). From such a library, one can select antibodies that bind the antigen used in immunization. See, for example, Vaccine. (2003) 22(2):257-67 or Immunogenetics. (2005) 57(10):730-8. Thus one could obtain primate antibodies that bind and inhibit plasma kallikrein by immunizing a chimpanzee or macaque and using a variety of means to select or screen for primate antibodies that bind and inhibit plasma kallikrein. One can also make chimeras of primatized Fabs with human constant regions, see Curr Opin Mol Ther. (2004) 6(6):675-83. "PRIMATIZED antibodies, genetically engineered from cynomolgus macaque monkey and human components, are structurally indistinguishable from human antibodies. They may, therefore, be less likely to cause adverse reactions in humans, making them potentially suited for long-term, chronic treatment" *Curr Opin Investig Drugs*. (2001) 2(5):635-8.

One exemplary type of library presents a diverse pool of polypeptides, each of which includes an immunoglobulin domain, e.g., an immunoglobulin variable domain. Of interest are display libraries where the members of the library include primate or "primatized" (e.g., such as human, non-human primate or "humanized") immunoglobin domains (e.g., immunoglobin variable domains) or chimeric primatized Fabs with human constant regions. Human or humanized immunoglobin domain libraries may be used to identify human or "humanized" antibodies that, for example, recognize human antigens. Because the constant and framework regions of the antibody are human, these antibodies may avoid themselves being recognized and targeted as antigens when administered to humans. The constant regions may also be optimized to recruit effector functions of the human immune system. The in vitro display selection process surmounts the inability of a normal human immune system to generate antibodies against self-antigens.

A typical antibody display library displays a polypeptide that includes a VH domain and a VL domain. An "immunoglobulin domain" refers to a domain from the variable or constant domain of immunoglobulin molecules Immunoglobulin domains typically contain two β-sheets formed of about seven β-strands, and a conserved disulphide bond (see, e.g., A. F. Williams and A. N. Barclay, 1988, Ann. Rev. Immunol. 6:381-405). The display library can display the antibody as a Fab fragment (e.g., using two polypeptide chains) or a single chain Fv (e.g., using a single polypeptide chain). Other formats can also be used.

As in the case of the Fab and other formats, the displayed antibody can include one or more constant regions as part of a light and/or heavy chain. In one embodiment, each chain includes one constant region, e.g., as in the case of a Fab. In other embodiments, additional constant regions are displayed.

Antibody libraries can be constructed by a number of processes (see, e.g., de Haard et al., 1999, J. Biol. Chem. 274: 18218-30; Hoogenboom et al., 1998, Immunotechnology 4:1-20; Hoogenboom et al., 2000, Immunol. Today 21:371-378, and Hoet et al. (2005) Nat Biotechnol. 23(3):344-8. Further, elements of each process can be combined with those of other processes. The processes can be used such that variation is introduced into a single immunoglobulin domain (e.g., VH or VL) or into multiple immunoglobulin domains (e.g., VH and VL). The variation can be introduced into an immunoglobulin variable domain, e.g., in the region of one or more of CDR1, CDR2, CDR3, FR1, FR2, FR3, and/or FR4, referring to such regions of either and both of heavy and light chain variable domains. For example, the variation(s) may be introduced into all three CDRs of a given variable domain, or into CDR1 and CDR2, e.g., of a heavy chain variable domain. Any combination is feasible. In one process, antibody libraries are constructed by inserting diverse oligonucleotides that encode CDRs into the corresponding regions of the nucleic acid. The oligonucleotides can be synthesized using monomeric nucleotides or trinucleotides. For example, Knappik et al., 2000, J. Mol. Biol. 296:57-86 describe a method for constructing CDR encoding oligonucleotides using trinucleotide synthesis and a template with engineered restriction sites for accepting the oligonucleotides.

In another process, an animal (e.g., a rodent) is immunized with plasma kallikrein. The animal is optionally boosted with the antigen to further stimulate the response. Then spleen cells are isolated from the animal, and nucleic acid encoding VH and/or VL domains is amplified and cloned for expression in the display library.

In yet another process, antibody libraries are constructed from nucleic acid amplified from naïve germline immunoglobulin genes. The amplified nucleic acid includes nucleic acid encoding the VH and/or VL domain. Sources of immunoglobulin-encoding nucleic acids are described below. Amplification can include PCR, e.g., with primers that anneal to the conserved constant region, or another amplification method.

Nucleic acid encoding immunoglobulin domains can be obtained from the immune cells of, e.g., a primate (e.g., a human), mouse, rabbit, camel, or rodent. In one example, the cells are selected for a particular property. B cells at various stages of maturity can be selected. In another example, the B cells are naïve.

In one embodiment, fluorescent-activated cell sorting (FACS) is used to sort B cells that express surface-bound IgM, IgD, or IgG molecules. Further, B cells expressing different isotypes of IgG can be isolated. In another preferred embodiment, the B or T cells are cultured in vitro. The cells can be stimulated in vitro, e.g., by culturing with feeder cells or by adding mitogens or other modulatory reagents, such as antibodies to CD40, CD40 ligand or CD20, phorbol myristate acetate, bacterial lipopolysaccharide, concanavalin A, phytohemagglutinin, or pokeweed mitogen.

In another embodiment, the cells are isolated from a subject that has a disease of condition described herein, e.g., a plasma kallikrein associated disease or condition.

In one preferred embodiment, the cells have activated a program of somatic hypermutation. Cells can be stimulated to undergo somatic mutagenesis of immunoglobulin genes, for example, by treatment with anti-immunoglobulin, anti-CD40, and anti-CD38 antibodies (see, e.g., Bergthorsdottir et al., 2001, J. Immunol. 166:2228). In another embodiment, the cells are naïve.

The nucleic acid encoding an immunoglobulin variable domain can be isolated from a natural repertoire by the following exemplary method. First, RNA is isolated from the immune cell. Full length (i.e., capped) mRNAs are separated (e.g. by degrading uncapped RNAs with calf intestinal phosphatase). The cap is then removed with tobacco acid pyrophosphatase and reverse transcription is used to produce the cDNAs.

The reverse transcription of the first (antisense) strand can be done in any manner with any suitable primer. See, e.g., de Haard et al., 1999, J. Biol. Chem. 274:18218-30. The primer binding region can be constant among different immunoglobulins, e.g., in order to reverse transcribe different isotypes of immunoglobulin. The primer binding region can also be specific to a particular isotype of immunoglobulin. Typically, the primer is specific for a region that is 3' to a sequence encoding at least one CDR. In another embodiment, poly-dT primers may be used (and may be preferred for the heavy-chain genes).

A synthetic sequence can be ligated to the 3' end of the reverse transcribed strand. The synthetic sequence can be used as a primer binding site for binding of the forward primer during PCR amplification after reverse transcription. The use of the synthetic sequence can obviate the need to use a pool of different forward primers to fully capture the available diversity.

The variable domain-encoding gene is then amplified, e.g., using one or more rounds. If multiple rounds are used, nested primers can be used for increased fidelity. The amplified nucleic acid is then cloned into a display library vector.

Secondary Screening Methods

After selecting candidate library members that bind to a target, each candidate library member can be further analyzed, e.g., to further characterize its binding properties for the target, e.g., plasma kallikrein. Each candidate library member can be subjected to one or more secondary screening assays. The assay can be for a binding property, a catalytic property, an inhibitory property, a physiological property (e.g., cytotoxicity, renal clearance, immunogenicity), a structural property (e.g., stability, conformation, oligomerization state) or another functional property. The same assay can be used repeatedly, but with varying conditions, e.g., to determine pH, ionic, or thermal sensitivities.

As appropriate, the assays can use a display library member directly, a recombinant polypeptide produced from the nucleic acid encoding the selected polypeptide, or a synthetic peptide synthesized based on the sequence of the selected polypeptide. In the case of selected Fabs, the Fabs can be evaluated or can be modified and produced as intact IgG proteins. Exemplary assays for binding properties include the following.

ELISA.

Binding proteins can be evaluated using an ELISA assay. For example, each protein is contacted to a microtitre plate whose bottom surface has been coated with the target, e.g., a limiting amount of the target. The plate is washed with buffer to remove non-specifically bound polypeptides. Then the amount of the binding protein bound to the target on the plate is determined by probing the plate with an antibody that can recognize the binding protein, e.g., a tag or constant portion of the binding protein. The antibody is linked to a detection system (e.g., an enzyme such as alkaline phosphatase or horse radish peroxidase (HRP) which produces a colorimetric product when appropriate substrates are provided).

Homogeneous Binding Assays.

The ability of a binding protein described herein to bind a target can be analyzed using a homogenous assay, i.e., after all components of the assay are added, additional fluid manipulations are not required. For example, fluorescence resonance energy transfer (FRET) can be used as a homogenous assay (see, for example, Lakowicz et al., U.S. Pat. No. 5,631,169; Stavrianopoulos, et al., U.S. Pat. No. 4,868,103). A fluorophore label on the first molecule (e.g., the molecule identified in the fraction) is selected such that its emitted fluorescent energy can be absorbed by a fluorescent label on a second molecule (e.g., the target) if the second molecule is in proximity to the first molecule. The fluorescent label on the second molecule fluoresces when it absorbs to the transferred energy. Since the efficiency of energy transfer between the labels is related to the distance separating the molecules, the spatial relationship between the molecules can be assessed. In a situation in which binding occurs between the molecules, the fluorescent emission of the 'acceptor' molecule label in the assay should be maximal. A binding event that is configured for monitoring by FRET can be conveniently measured through standard fluorometric detection means, e.g., using a fluorimeter. By titrating the amount of the first or second binding molecule, a binding curve can be generated to estimate the equilibrium binding constant.

Another example of a homogenous assay is ALPHASCREEN™ (Packard Bioscience, Meriden Conn.). ALPHASCREEN™ uses two labeled beads. One bead generates singlet oxygen when excited by a laser. The other bead generates a light signal when singlet oxygen diffuses from the first bead and collides with it. The signal is only generated when the two beads are in proximity. One bead can be attached to the display library member, the other to the target. Signals are measured to determine the extent of binding.

Surface Plasmon Resonance (SPR).

The interaction of binding protein and a target can be analyzed using SPR. SPR or Biomolecular Interaction Analysis (BIA) detects biospecific interactions in real time, without labeling any of the interactants. Changes in the mass at the binding surface (indicative of a binding event) of the BIA chip result in alterations of the refractive index of light near the surface (the optical phenomenon of surface plasmon resonance (SPR)). The changes in the refractivity generate a detectable signal, which are measured as an indication of real-time reactions between biological molecules. Methods for using SPR are described, for example, in U.S. Pat. No. 5,641,640; Raether, 1988, Surface Plasmons Springer Verlag; Sjolander and Urbaniczky, 1991, Anal. Chem. 63:2338-2345; Szabo et al., 1995, Curr. Opin. Struct. Biol. 5:699-705 and on-line resources provide by BIAcore International AB (Uppsala, Sweden).

Information from SPR can be used to provide an accurate and quantitative measure of the equilibrium dissociation constant (K_D), and kinetic parameters, including K_{on} and K_{off}, for the binding of a binding protein to a target. Such data can be used to compare different biomolecules. For example, selected proteins from an expression library can be compared to identify proteins that have high affinity for the target or that have a slow K_{off}. This information can also be used to develop structure-activity relationships (SAR). For example, the kinetic and equilibrium binding parameters of matured versions of a parent protein can be compared to the parameters of the parent protein. Variant amino acids at given positions can be identified that correlate with particular binding parameters, e.g., high affinity and slow K_{off}. This information can be combined with structural modeling (e.g., using homology modeling, energy minimization, or structure determination by x-ray crystallography or NMR). As a result, an understanding of the physical interaction between the protein and its target can be formulated and used to guide other design processes.

Cellular Assays.

Binding proteins can be screened for ability to bind to cells which transiently or stably express and display the target of interest on the cell surface. For example, plasma kallikrein binding proteins can be fluorescently labeled and binding to plasma kallikrein in the presence of absence of antagonistic antibody can be detected by a change in fluorescence intensity using flow cytometry e.g., a FACS machine.

Other Exemplary Methods for Obtaining Plasma Kallikrein Binding Proteins

In addition to the use of display libraries, other methods can be used to obtain a plasma kallikrein binding protein (e.g., antibody). For example, plasma kallikrein protein or a fragment thereof can be used as an antigen in a non-human animal, e.g., a rodent.

In one embodiment, the non-human animal includes at least a part of a human immunoglobulin gene. For example, it is possible to engineer mouse strains deficient in mouse antibody production with large fragments of the human Ig loci. Using the hybridoma technology, antigen-specific monoclonal antibodies (Mabs) derived from the genes with the desired specificity may be produced and selected. See, e.g., XENOMOUSE™, Green et al., 1994, *Nat. Gen.* 7:13-21; U.S. 2003-0070185, WO 96/34096, published Oct. 31, 1996, and PCT Application No. PCT/US96/05928, filed Apr. 29, 1996.

In another embodiment, a monoclonal antibody is obtained from the non-human animal, and then modified, e.g., humanized or deimmunized. Winter describes a CDR-grafting method that may be used to prepare the humanized antibodies (UK Patent Application GB 2188638A, filed on Mar. 26, 1987; U.S. Pat. No. 5,225,539. All of the CDRs of a particular human antibody may be replaced with at least a portion of a non-human CDR or only some of the CDRs may be replaced with non-human CDRs. It is only necessary to replace the number of CDRs required for binding of the humanized antibody to a predetermined antigen.

Humanized antibodies can be generated by replacing sequences of the Fv variable region that are not directly involved in antigen binding with equivalent sequences from human Fv variable regions. General methods for generating humanized antibodies are provided by Morrison, S. L., 1985, Science 229:1202-1207, by Oi et al., 1986, BioTechniques 4:214, and by Queen et al. U.S. Pat. Nos. 5,585,089, 5,693,761 and 5,693,762. Those methods include isolating, manipulating, and expressing the nucleic acid sequences that encode all or part of immunoglobulin Fv variable regions from at least one of a heavy or light chain. Numerous sources of such nucleic acid are available. For example, nucleic acids may be obtained from a hybridoma producing an antibody against a predetermined target, as described above. The recombinant DNA encoding the humanized antibody, or fragment thereof, can then be cloned into an appropriate expression vector.

Reducing Immunogenicity of Plasma Kallikrein Binding Proteins

Immunoglobin plasma kallikrein binding proteins (e.g., IgG or Fab plasma kallikrein binding proteins) may be modified to reduce immunogenicity. Reduced immunogenicity is desirable in plasma kallikrein binding proteins intended for use as therapeutics, as it reduces the chance that the subject will develop an immune response against the therapeutic molecule. Techniques useful for reducing immunogenicity of plasma kallikrein binding proteins include deletion/modification of potential human T cell epitopes and "germlining" of sequences outside of the CDRs (e.g., framework and Fc).

A plasma kallikrein-binding antibody may be modified by specific deletion of human T cell epitopes or "deimmunization," e.g., by the methods disclosed in WO 98/52976 and WO 00/34317. Briefly, the heavy and light chain variable regions of an antibody are analyzed for peptides that bind to MHC Class II; these peptides represent potential T-cell epitopes (as defined in WO 98/52976 and WO 00/34317). For detection of potential T-cell epitopes, a computer modeling approach termed "peptide threading" can be applied, and in addition a database of human MHC class II binding peptides can be searched for motifs present in the VH and VL sequences, as described in WO 98/52976 and WO 00/34317. These motifs bind to any of the 18 major MHC class II DR allotypes, and thus constitute potential T cell epitopes. Potential T-cell epitopes detected can be eliminated by substituting small numbers of amino acid residues in the variable regions, or preferably, by single amino acid substitutions. As far as possible conservative substitutions are made, often but not exclusively, an amino acid common at this position in human germline antibody sequences may be used. Human germline sequences are disclosed in Tomlinson, I. A. et al., 1992, J. Mol. Biol. 227:776-798; Cook, G. P. et al., 1995, Immunol. Today Vol. 16 (5): 237-242; Chothia, D. et al., 1992, J. Mol. Bio. 227:799-817. The V BASE directory provides a comprehensive directory of human immunoglobulin variable region sequences (compiled by Tomlinson, I. A. et al. MRC Centre for Protein Engineering, Cambridge, UK). After the deimmunizing changes are identified, nucleic acids encoding V_H and V_L can be constructed by mutagenesis or other synthetic methods (e.g., de novo synthesis, cassette replacement, and so forth). Mutagenized variable sequence can, optionally, be fused to a human constant region, e.g., human IgG1 or κ constant regions.

In some cases a potential T cell epitope will include residues which are known or predicted to be important for antibody function. For example, potential T cell epitopes are usually biased towards the CDRs. In addition, potential T cell epitopes can occur in framework residues important for antibody structure and binding. Changes to eliminate these potential epitopes will in some cases require more scrutiny, e.g., by making and testing chains with and without the change. Where possible, potential T cell epitopes that overlap the CDRs were eliminated by substitutions outside the CDRs. In some cases, an alteration within a CDR is the only option, and thus variants with and without this substitution should be tested. In other cases, the substitution required to remove a potential T cell epitope is at a residue position within the framework that might be critical for antibody binding. In these cases, variants with and without this substitution should be tested. Thus, in some cases several variant deimmunized heavy and light chain variable regions were designed and various heavy/light chain combinations tested in order to identify the optimal deimmunized antibody. The choice of the final deimmunized antibody can then be made by considering the binding affinity of the different variants in conjunction with the extent of deimmunization, i.e., the number of potential T cell epitopes remaining in the variable region. Deimmunization can be used to modify any antibody, e.g., an antibody that includes a non-human sequence, e.g., a synthetic antibody, a murine antibody other non-human monoclonal antibody, or an antibody isolated from a display library.

Plasma kallikrein binding antibodies are "germlined" by reverting one or more non-germline amino acids in framework regions to corresponding germline amino acids of the antibody, so long as binding properties are substantially retained. Similar methods can also be used in the constant region, e.g., in constant immunoglobulin domains.

Antibodies that bind to plasma kallikrein, e.g., an antibody described herein, may be modified in order to make the variable regions of the antibody more similar to one or more germline sequences. For example, an antibody can include one, two, three, or more amino acid substitutions, e.g., in a framework, CDR, or constant region, to make it more similar to a reference germline sequence. One exemplary germlining method can include identifying one or more germline sequences that are similar (e.g., most similar in a particular database) to the sequence of the isolated antibody. Mutations (at the amino acid level) are then made in the isolated antibody, either incrementally or in combination with other mutations. For example, a nucleic acid library that includes sequences encoding some or all possible germline mutations is made. The mutated antibodies are then evaluated, e.g., to identify an antibody that has one or more additional germline residues relative to the isolated antibody and that is still useful (e.g., has a functional activity). In one embodiment, as many germline residues are introduced into an isolated antibody as possible.

In one embodiment, mutagenesis is used to substitute or insert one or more germline residues into a framework and/or constant region. For example, a germline framework and/or constant region residue can be from a germline sequence that is similar (e.g., most similar) to the non-variable region being modified. After mutagenesis, activity (e.g., binding or other functional activity) of the antibody can be evaluated to determine if the germline residue or residues are tolerated (i.e., do not abrogate activity). Similar mutagenesis can be performed in the framework regions.

Selecting a germline sequence can be performed in different ways. For example, a germline sequence can be selected if it meets a predetermined criteria for selectivity or similarity, e.g., at least a certain percentage identity, e.g., at least 75, 80, 85, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, or 99.5% identity. The selection can be performed using at least 2, 3, 5, or 10 germline sequences. In the case of CDR1 and CDR2, identifying a similar germline sequence can include selecting one such sequence. In the case of CDR3, identifying a similar germline sequence can include selecting one such sequence, but may include using two germline sequences that separately contribute to the amino-terminal portion and the carboxy-terminal portion of the sequence. In other implementations more than one or two germline sequences are used, e.g., to form a consensus sequence.

In one embodiment, with respect to a particular reference variable domain sequence, e.g., a sequence described herein, a related variable domain sequence has at least 30, 40, 50, 60, 70, 80, 90, 95 or 100% of the CDR amino acid positions that are not identical to residues in the reference CDR sequences, residues that are identical to residues at corresponding positions in a human germline sequence (i.e., an amino acid sequence encoded by a human germline nucleic acid).

In one embodiment, with respect to a particular reference variable domain sequence, e.g., a sequence described herein, a related variable domain sequence has at least 30, 50, 60, 70, 80, 90 or 100% of the FR regions identical to FR sequence from a human germline sequence, e.g., a germline sequence related to the reference variable domain sequence.

Accordingly, it is possible to isolate an antibody which has similar activity to a given antibody of interest, but is more similar to one or more germline sequences, particularly one or more human germline sequences. For example, an antibody can be at least 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, or 99.5% identical to a germline sequence in a region outside the CDRs (e.g., framework regions). Further, an antibody can include at least 1, 2, 3, 4, or 5 germline residues in a CDR region, the germline residue being from a germline sequence of similar (e.g., most similar) to the variable region being modified. Germline sequences of primary interest are human germline sequences. The activity of the antibody (e.g., the binding activity as measured by K_A) can be within a factor or 100, 10, 5, 2, 0.5, 0.1, and 0.001 of the original antibody.

Germline sequences of human immunoglobin genes have been determined and are available from a number of sources, including the INTERNATIONAL IMMUNOGENETICS INFORMATION SYSTEM® (IMGT), and the V BASE directory (compiled by Tomlinson, I. A. et al. MRC Centre for Protein Engineering, Cambridge, UK).

Exemplary germline reference sequences for V_{kappa} include: O12/O2, O18/O8, A20, A30, L14, L1, L15, L4/18a, L5/L19, L8, L23, L9, L24, L11, L12, O11/O1, A17, A1, A18, A2, A19/A3, A23, A27, A11, L2/L16, L6, L20, L25, B3, B2, A26/A10, and A14. See, e.g., Tomlinson et al., 1995, EMBO J. 14(18):4628-3.

A germline reference sequence for the HC variable domain can be based on a sequence that has particular canonical structures, e.g., 1-3 structures in the H1 and H2 hypervariable loops. The canonical structures of hypervariable loops of an immunoglobulin variable domain can be inferred from its sequence, as described in Chothia et al., 1992, J. Mol. Biol. 227:799-817; Tomlinson et al., 1992, J. Mol. Biol. 227:776-798); and Tomlinson et al., 1995, EMBO J. 14(18):4628-38. Exemplary sequences with a 1-3 structure include: DP-1, DP-8, DP-12, DP-2, DP-25, DP-15, DP-7, DP-4, DP-31, DP-32, DP-33, DP-35, DP-40, 7-2, hv3005, hv3005f3, DP-46, DP-47, DP-58, DP-49, DP-50, DP-51, DP-53, and DP-54.

Protein Production

Standard recombinant nucleic acid methods can be used to express a protein that binds to plasma kallikrein. Generally, a nucleic acid sequence encoding the protein is cloned into a nucleic acid expression vector. Of course, if the protein includes multiple polypeptide chains, each chain can be cloned into an expression vector, e.g., the same or different vectors, that are expressed in the same or different cells.

Antibody Production.

Some antibodies, e.g., Fabs, can be produced in bacterial cells, e.g., *E. coli* cells (see e.g., Nadkarni, A. et al., 2007 Protein Expr Purif 52(1):219-29). For example, if the Fab is encoded by sequences in a phage display vector that includes a suppressible stop codon between the display entity and a bacteriophage protein (or fragment thereof), the vector nucleic acid can be transferred into a bacterial cell that cannot suppress a stop codon. In this case, the Fab is not fused to the gene III protein and is secreted into the periplasm and/or media.

Antibodies can also be produced in eukaryotic cells. In one embodiment, the antibodies (e.g., scFv's) are expressed in a yeast cell such as *Pichia* (see, e.g., Powers et al., 2001, J. Immunol. Methods. 251:123-35; Schoonooghe S. et al., 2009 BMC Biotechnol. 9:70; Abdel-Salam, H A. et al., 2001 Appl Microbiol Biotechnol 56(1-2):157-64; Takahashi K. et al., 2000 Biosci Biotechnol Biochem 64(10):2138-44; Edqvist, J. et al., 1991 J Biotechnol 20(3):291-300), *Hanseula*, or *Saccharomyces*. One of skill in the art can optimize antibody production in yeast by optimizing, for example, oxygen conditions (see e.g., Baumann K., et al. 2010 BMC Syst. Biol. 4:141), osmolarity (see e.g., Dragosits, M. et al., 2010 BMC Genomics 11:207), temperature (see e.g., Dragosits, M. et al., 2009 J Proteome Res. 8(3):1380-92), fermentation conditions (see e.g., Ning, D. et al. 2005 J. Biochem. and Mol. Biol. 38(3): 294-299), strain of yeast (see e.g., Kozyr, A V et al. 2004 Mol Biol (Mosk) 38(6):1067-75; Horwitz, A H. et al., 1988 Proc Natl Acad Sci USA 85(22):8678-82; Bowdish, K. et al. 1991 J Biol Chem 266(18):11901-8), overexpression of proteins to enhance antibody production (see e.g., Gasser, B. et al., 2006 Biotechol. Bioeng. 94(2):353-61), level of acidity of the culture (see e.g., Kobayashi H., et al., 1997 FEMS Microbiol Lett 152(2):235-42), concentrations of substrates and/or ions (see e.g., Ko J H. et al., 2996 Appl Biochem Biotechnol 60(1):41-8). In addition, yeast systems can be used to produce antibodies with an extended half-life (see e.g., Smith, B J. et al. 2001 Bioconjug Chem 12(5):750-756),

In one preferred embodiment, antibodies are produced in mammalian cells. Preferred mammalian host cells for expressing the clone antibodies or antigen-binding fragments thereof include Chinese Hamster Ovary (CHO cells) (including dhfr-CHO cells, described in Urlaub and Chasin, 1980, Proc. Natl. Acad. Sci. USA 77:4216-4220, used with a DHFR selectable marker, e.g., as described in Kaufman and Sharp, 1982, Mol. Biol. 159:601 621), lymphocytic cell lines, e.g., NS0 myeloma cells and SP2 cells, COS cells, HEK293T cells (J. Immunol. Methods (2004) 289(1-2):65-80), and a cell from a transgenic animal, e.g., a transgenic mammal. For example, the cell is a mammary epithelial cell.

In some embodiments, plasma kallikrein binding proteins are produced in a plant or cell-free based system (see e.g., Galeffi, P., et al., 2006 J Transl Med 4:39).

In addition to the nucleic acid sequence encoding the diversified immunoglobulin domain, the recombinant expression vectors may carry additional sequences, such as sequences that regulate replication of the vector in host cells (e.g., origins of replication) and selectable marker genes. The selectable marker gene facilitates selection of host cells into which the vector has been introduced (see e.g., U.S. Pat. Nos. 4,399,216, 4,634,665 and 5,179,017). For example, typically the selectable marker gene confers resistance to drugs, such as G418, hygromycin or methotrexate, on a host cell into which the vector has been introduced. Preferred selectable marker genes include the dihydrofolate reductase (DHFR) gene (for use in $dhfr^-$ host cells with methotrexate selection/amplification) and the neo gene (for G418 selection).

In an exemplary system for recombinant expression of an antibody, or antigen-binding portion thereof, a recombinant expression vector encoding both the antibody heavy chain and the antibody light chain is introduced into dhfr CHO cells by calcium phosphate-mediated transfection. Within the recombinant expression vector, the antibody heavy and light chain genes are each operatively linked to enhancer/promoter regulatory elements (e.g., derived from SV40, CMV, adenovirus and the like, such as a CMV enhancer/AdMLP promoter regulatory element or an SV40 enhancer/AdMLP promoter regulatory element) to drive high levels of transcription of the genes. The recombinant expression vector also carries a DHFR gene, which allows for selection of CHO cells that have been transfected with the vector using methotrexate selection/amplification. The selected transformant host cells are cultured to allow for expression of the antibody heavy and light chains and intact antibody is recovered from the culture medium Standard molecular biology techniques are used to prepare the recombinant expression vector, transfect the host cells, select for transformants, culture the host cells and recover the antibody from the culture medium. For example, some antibodies can be isolated by affinity chromatography with a Protein A or Protein G coupled matrix.

For antibodies that include an Fc domain, the antibody production system may produce antibodies in which the Fc region is glycosylated. For example, the Fc domain of IgG molecules is glycosylated at asparagine 297 in the CH2 domain. This asparagine is the site for modification with biantennary-type oligosaccharides. It has been demonstrated that this glycosylation is required for effector functions mediated by Fcg receptors and complement C1q (Burton and Woof, 1992, Adv. Immunol. 51:1-84; Jefferis et al., 1998, Immunol. Rev. 163:59-76). In one embodiment, the Fc domain is produced in a mammalian expression system that appropriately glycosylates the residue corresponding to asparagine 297. The Fc domain can also include other eukaryotic post-translational modifications.

Antibodies can also be produced by a transgenic animal. For example, U.S. Pat. No. 5,849,992 describes a method of expressing an antibody in the mammary gland of a transgenic mammal. A transgene is constructed that includes a milk-specific promoter and nucleic acids encoding the antibody of interest and a signal sequence for secretion. The milk produced by females of such transgenic mammals includes, secreted-therein, the antibody of interest. The antibody can be purified from the milk, or for some applications, used directly.

Characterization of Plasma Kallikrein Binding Proteins

IC_{50} (Inhibitory Concentration 50%) and EC_{50} (Effective Concentration 50%).

Within a series or group of binding proteins, those having lower IC_{50} or EC_{50} values are considered more potent inhibitors of plasma kallikrein than those binding proteins having higher IC_{50} or EC_{50} values. Exemplary binding proteins have an IC_{50} value of less than 800 nM, 400 nM, 100 nM, 25 nM, 5 nM, or 1 nM, e.g., as measured in an in vitro assay for inhibition of plasma kallikrein activity when the plasma kallikrein is at 2 μM.

Plasma kallikrein binding proteins may also be characterized with reference to the activity of Factor XII and HMWK (high-molecular-weight kininogen) signaling events, e.g., the production of Factor XIIa and/or bradykinin.

The binding proteins can also be evaluated for selectivity toward plasma kallikrein. For example, a plasma kallikrein binding protein can be assayed for its potency toward plasma kallikrein and a panel of kallikreins and an IC_{50} value or EC_{50} value can be determined for each kallikrein. In one embodiment, a compound that demonstrates a low IC_{50} value or EC_{50} value for the plasma kallikrein, and a higher IC_{50} value or EC_{50} value, e.g., at least 2-, 5-, or 10-fold higher, for another kallikrein within the test panel is considered to be selective toward plasma kallikrein.

A pharmacokinetics study in rat, mice, or monkey can be performed with plasma kallikrein binding proteins for determining plasma kallikrein half-life in the serum. Likewise, the effect of the binding protein can be assessed in vivo, e.g., in an animal model for a disease (e.g., carrageenin-induced edema in rat hind paw (Winter et al. Proc Soc Exp Biol Med. 1962; 111:544-7)), for use as a therapeutic, for example, to treat a disease or condition described herein, e.g., a plasma kallikrein associated disorder.

Pharmaceutical Compositions

Proteins (e.g., binding proteins) that bind to plasma kallikrein (e.g., human plasma kallikrein and/or murine plasma kallikrein) and, e.g., include at least one immunoglobin variable region can be used in methods for treating (or preventing) a plasma kallikrein associated disease or condition. The binding proteins can be present in a composition, e.g., a pharmaceutically acceptable composition or pharmaceutical composition, which includes a plasma kallikrein-binding protein, e.g., an antibody molecule or other polypeptide or peptide identified as binding to plasma kallikrein, as described herein. The plasma kallikrein binding protein can be formulated together with a pharmaceutically acceptable carrier. Pharmaceutical compositions include therapeutic compositions and diagnostic compositions, e.g., compositions that include labeled plasma kallikrein binding proteins for in vivo imaging, and compositions that include labeled plasma kallikrein binding proteins for treating (or preventing) a plasma kallikrein associated disease.

A pharmaceutically acceptable carrier includes any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents, and the like that are physiologically compatible. Preferably, the carrier is suitable for intravenous, intramuscular, subcutaneous, parenteral, spinal, or epidermal administration (e.g., by injection or infusion), although carriers suitable for inhalation and intranasal administration are also contemplated. Depending on the route of administration, the plasma kallikrein binding protein may be coated in a material to protect the compound from the action of acids and other natural conditions that may inactivate the compound.

A pharmaceutically acceptable salt is a salt that retains the desired biological activity of the compound and does not impart any undesired toxicological effects (see e.g., Berge, S. M., et al., 1977, J. Pharm. Sci. 66:1-19). Examples of such salts include acid addition salts and base addition salts. Acid addition salts include those derived from nontoxic inorganic acids, such as hydrochloric, nitric, phosphoric, sulfuric, hydrobromic, hydroiodic, phosphorous, and the like, as well as from nontoxic organic acids such as aliphatic mono- and dicarboxylic acids, phenyl-substituted alkanoic acids, hydroxy alkanoic acids, aromatic acids, aliphatic and aromatic sulfonic acids, and the like. Base addition salts include those derived from alkaline earth metals, such as sodium, potassium, magnesium, calcium, and the like, as well as from nontoxic organic amines, such as N,N'-dibenzylethylenediamine, N-methylglucamine, chloroprocaine, choline, diethanolamine, ethylenediamine, procaine, and the like.

The compositions may be in a variety of forms. These include, for example, liquid, semi-solid and solid dosage forms, such as liquid solutions (e.g., injectable and infusible solutions), dispersions or suspensions, tablets, pills, powders, liposomes and suppositories. The form can depend on the intended mode of administration and therapeutic application. Many compositions are in the form of injectable or infusible solutions, such as compositions similar to those used for administration of humans with antibodies. An exemplary mode of administration is parenteral (e.g., intravenous, subcutaneous, intraperitoneal, intramuscular). In one embodiment, the plasma kallikrein binding protein is administered by intravenous infusion or injection. In another preferred embodiment, the plasma kallikrein binding protein is administered by intramuscular or subcutaneous injection.

The phrases "parenteral administration" and "administered parenterally" as used herein means modes of administration other than enteral and topical administration, usually by injection, and includes, without limitation, intravenous, intramuscular, intraarterial, intrathecal, intracapsular, intraorbital, intracardiac, intradermal, intraperitoneal, transtracheal, subcutaneous, subcuticular, intraarticular, subcapsular, subarachnoid, intraspinal, epidural and intrasternal injection and infusion.

The composition can be formulated as a solution, microemulsion, dispersion, liposome, or other ordered structure suitable to high drug concentration. Sterile injectable solutions can be prepared by incorporating the binding protein in the required amount in an appropriate solvent with one or a combination of ingredients enumerated above, as required, followed by filtered sterilization. Generally, dispersions are prepared by incorporating the active compound into a sterile vehicle that contains a basic dispersion medium and the required other ingredients from those enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, the preferred methods of preparation are vacuum drying and freeze-drying that yields a powder of the active ingredient plus any additional desired ingredient from a previously sterile-filtered solution thereof. The proper fluidity of a solution can be maintained, for example, by the use of a coating such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of surfactants. Prolonged absorption of injectable compositions can be brought about by including in the composition an agent that delays absorption, for example, monostearate salts and gelatin.

A plasma kallikrein binding protein can be administered by a variety of methods, although for many applications, the preferred route/mode of administration is intravenous injection or infusion. For example, for therapeutic applications, the plasma kallikrein binding protein can be administered by intravenous infusion at a rate of less than 30, 20, 10, 5, or 1 mg/min to reach a dose of about 1 to 100 mg/m^2 or 7 to 25 mg/m^2. The route and/or mode of administration will vary depending upon the desired results. In certain embodiments, the active compound may be prepared with a carrier that will protect the compound against rapid release, such as a controlled release formulation, including implants, and microencapsulated delivery systems. Biodegradable, biocompatible polymers can be used, such as ethylene vinyl acetate, polyanhydrides, polyglycolic acid, collagen, polyorthoesters, and polylactic acid. Many methods for the preparation of such formulations are available. See, e.g., Sustained and Controlled Release Drug Delivery Systems, J. R. Robinson, ed., 1978, Marcel Dekker, Inc., New York.

Pharmaceutical compositions can be administered with medical devices. For example, in one embodiment, a pharmaceutical composition disclosed herein can be administered with a device, e.g., a needleless hypodermic injection device, a pump, or implant.

In certain embodiments, a plasma kallikrein binding protein can be formulated to ensure proper distribution in vivo. For example, the blood-brain barrier (BBB) excludes many highly hydrophilic compounds. To ensure that the therapeutic compounds disclosed herein cross the BBB (if desired), they can be formulated, for example, in liposomes. For methods of manufacturing liposomes, see, e.g., U.S. Pat. Nos. 4,522,811; 5,374,548; and 5,399,331. The liposomes may comprise one or more moieties that are selectively transported into specific cells or organs, thus enhance targeted drug delivery (see, e.g., V. V. Ranade, 1989, J. Clin. Pharmacol. 29:685).

Dosage regimens are adjusted to provide the optimum desired response (e.g., a therapeutic response). For example, a single bolus may be administered, several divided doses may be administered over time or the dose may be proportionally reduced or increased as indicated by the exigencies of the therapeutic situation. It is especially advantageous to formulate parenteral compositions in dosage unit form for ease of administration and uniformity of dosage. Dosage unit form as used herein refers to physically discrete units suited as unitary dosages for the subjects to be treated; each unit contains a predetermined quantity of active compound calculated to produce the desired therapeutic effect in association with the required pharmaceutical carrier. The specification for the dosage unit forms can be dictated by and directly dependent on (a) the unique characteristics of the active compound and the particular therapeutic effect to be achieved, and (b) the limitations inherent in the art of compounding such an active compound for the treatment of sensitivity in individuals.

An exemplary, non-limiting range for a therapeutically or prophylactically effective amount of a binding protein (e.g., an antibody) disclosed herein is 0.1-20 mg/kg, more preferably 1-10 mg/kg. An anti-plasma kallikrein antibody can be administered, e.g., by intravenous infusion, e.g., at a rate of less than 30, 20, 10, 5, or 1 mg/min to reach a dose of about 1 to 100 mg/m^2 or about 5 to 30 mg/m^2. For binding proteins smaller in molecular weight than an antibody, appropriate amounts can be proportionally less. Dosage values may vary with the type and severity of the condition to be alleviated. For a particular subject, specific dosage regimens can be adjusted over time according to the individual need and the professional judgment of the person administering or supervising the administration of the compositions.

The pharmaceutical compositions disclosed herein may include a "therapeutically effective amount" or a "prophylactically effective amount" of a plasma kallikrein binding protein disclosed herein. A "therapeutically effective amount" refers to an amount effective, at dosages and for periods of time necessary, to achieve the desired therapeutic result. A therapeutically effective amount of the composition may vary according to factors such as the disease state, age, sex, and weight of the individual, and the ability of the protein to elicit a desired response in the individual. A therapeutically effective amount is also one in which any toxic or detrimental effects of the composition are outweighed by the therapeutically beneficial effects.

A "therapeutically effective dosage" preferably modulates a measurable parameter, e.g., levels of circulating IgG antibodies by a statistically significant degree or at least about 20%, more preferably by at least about 40%, even more preferably by at least about 60%, and still more preferably by at least about 80% relative to untreated subjects. The ability of a compound to modulate a measurable parameter, e.g., a disease-associated parameter, can be evaluated in an animal model system predictive of efficacy in human disorders and conditions, e.g., a plasma kallikrein associated disease. Alternatively, this property of a composition can be evaluated by examining the ability of the compound to modulate a parameter in vitro.

A "prophylactically effective amount" refers to an amount effective, at dosages and for periods of time necessary, to achieve the desired prophylactic result. Typically, because a prophylactic dose is used in subjects prior to or at an earlier stage of disease, the prophylactically effective amount will be less than the therapeutically effective amount.

Stabilization and Retention

In one embodiment, a plasma kallikrein binding protein is physically associated with a moiety that improves its stabilization and/or retention in circulation, e.g., in blood, serum, lymph, or other tissues, e.g., by at least 1.5, 2, 5, 10, or 50 fold. For example, a plasma kallikrein binding protein can be associated with a polymer, e.g., a substantially non-antigenic polymer, such as polyalkylene oxides or polyethylene oxides. Suitable polymers will vary substantially by weight. Polymers having molecular number average weights ranging from about 200 to about 35,000 (or about 1,000 to about 15,000, and 2,000 to about 12,500) can be used. For example, a plasma kallikrein binding protein can be conjugated to a water soluble polymer, e.g., hydrophilic polyvinyl polymers, e.g., polyvinylalcohol and polyvinylpyrrolidone. A non-limiting list of such polymers include polyalkylene oxide homopolymers such as polyethylene glycol (PEG) or polypropylene glycols, polyoxyethylenated polyols, copolymers thereof and block copolymers thereof, provided that the water solubility of the block copolymers is maintained.

A plasma kallikrein binding protein can also be associated with a carrier protein, e.g., a serum albumin, such as a human serum albumin (see e.g., Smith, B J. et al., 2001 Bioconjug Chem 12(5): 750-756). For example, a translational fusion can be used to associate the carrier protein with the plasma kallikrein binding protein.

A plasma kallikrein binding protein can also be modified as a HESylation derivative. Processes for HESylation of a plasma kallikrein binding protein utilize hydroxyethyl starch to modify the protein. HESylation of a protein can extend the circulating half-life of the protein and also reduce renal clearance.

In some embodiments, the plasma kallikrein binding proteins as described herein are fused to an unstructured recombinant polymer (URP) (see e.g., U.S. Pat. No. 7,846,445, the contents of which are incorporated herein by reference in its entirety).

URPs are polypeptides composed of Gly, Ala, Ser, Thr, Glu, and Pro that have no secondary structure. In aqueous solvents, URPs are highly solvated and give the protein they are attached to an apparent molecular mass that is much larger than that of the polypeptide alone. A URP sequence can be fused to a plasma kallikrein binding protein to (i) increase circulating half-life, (ii) improve tissue selectivity, (iii) protect the binding protein from degradation, (iv) reduce immunogenicity, (v) interrupt T-cell epitopes, (vi) enhance solubility, (vii) improve pH profile and homogeneity of protein charge, (viii) improve purification properties due to a sharper pKa, (ix) improve formulation and delivery, and (x) improve protein production (see e.g., U.S. Pat. No. 7,846,445, which is incorporated herein by reference in its entirety).

In general, a URP sequence should be designed such that it lacks unintended activities such as interactions with serum proteins (e.g., antibodies). One of skill in the art can test a URP for unintended activities using e.g., an ELISA assay to detect the level of binding to an immobilized serum protein. In some embodiments, it may be desirable for a URP to interact with a serum protein (e.g., albumin) to increase the circulating half-life of the plasma kallikrein binding protein.

In general, it is desired that URP sequences behave like denatured peptide sequences under physiological conditions and as such, lack well defined secondary and tertiary structures under physiological conditions. Methods to ascertain the second and tertiary structures of a given polypeptide are known to those of skill in the art and include, but are not limited to, CD spectroscopy in the "far-UV" spectral region (190-250 nm), and computer programs or algorithms such as the Chou-Fasman algorithm (Chou, P. Y., et al. (1974) Biochemistry, 13: 222-45). URP sequences typically have a high degree of conformational flexibility under physiological conditions (e.g., pH 6.5-7.8 and 30-37° C.) and also have large hydrodynamic radii (Stokes' radius) compared to globular proteins of similar molecular weight.

In one embodiment, the URP sequences have low immunogenicity. Preferred URPs are designed to avoid formation of conformational epitopes. For example, of particular interest are URP sequences having a low tendency to adapt compactly folded conformations in aqueous solution. In particular, low immunogenicity can be achieved by choosing sequences that resist antigen processing in antigen presenting cells, choosing sequences that do not bind MHC well and/or by choosing sequences that are derived from host (e.g., human) sequences.

In some embodiments, the URP sequences have a high degree of protease resistance to extend serum half-life. URPs can also be characterized by the effect they have on a protein sequence e.g., the protein exhibits a longer serum half-life and/or higher solubility as compared to the corresponding protein that is deficient in the URP. Methods of ascertaining serum half-life are known in the art (see e.g., Alvarez, P., et al. (2004) J Biol Chem, 279: 3375-81). One can readily determine whether the resulting protein has a longer serum half-life as compared to the unmodified protein by practicing any methods available in the art or exemplified herein.

The URP can be of any length necessary to effect (a) extension of serum half-life of a protein comprising the URP; (b) an increase in solubility of the resulting protein; (c) an increased resistance to protease; and/or (d) a reduced immunogenicity of the resulting protein that comprises the URP. In some embodiments, the URP has about 30, 40, 50, 60, 70, 80, 90, 100, 150, 200, 300, 400 or more contiguous amino acids. When incorporated into a protein, the URP can be fragmented such that the resulting protein contains multiple URPs, or multiple fragments of URPs. Some or all of these individual URP sequences may be shorter than 40 amino acids, provided that the combined length of all URP sequences in the resulting protein is at least 40 amino acids. Preferably, the resulting protein has a combined length of URP sequences exceeding 40, 50, 60, 70, 80, 90, 100, 150, 200 or more amino acids.

In some embodiments, the isoelectric point (pI) of the URP is 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 4.5, 5.0, 5.5, 6.0, 6.5, 7.0, 7.5, 8.0, 8.5, 9.0, 9.5, 10.0, 10.5, 11.0, 11.5, 12.0, 12.5 or even 13.0.

In general, URP sequences are rich in hydrophilic amino acids and contain a low percentage of hydrophobic or aromatic amino acids. Suitable hydrophilic residues include but are not limited to glycine, serine, aspartate, glutamate, lysine, arginine, and threonine. Hydrophobic residues that are less favored in construction of URPs include tryptophan, phenylalanine, tyrosine, leucine, isoleucine, valine, and methionine. URP sequences can be rich in glycine but URP sequences can also be rich in the amino acids glutamate, aspartate, serine, threonine, alanine or proline. Thus the predominant amino acid may be G, E, D, S, T, A or P. The inclusion of proline residues tends to reduce sensitivity to proteolytic degradation.

In some embodiments, the URP sequences include hydrophilic residues to increase their solubility in water and aqueous media under physiological conditions. The inclusion of hydrophilic residues reduces the formation of aggregates in aqueous formulations and the fusion of URP sequences to other proteins or peptides (e.g., a plasma kallikrein binding protein) can enhance their solubility and reduce aggregate formation and immunogenicity.

URP sequences can be further designed to avoid amino acids that confer undesirable properties to the protein, for example, cysteine (to avoid disulfide formation and oxidation), methionine (to avoid oxidation), asparagine and glutamine (to avoid desamidation).

In some embodiments, a URP is designed to be glycine-rich (e.g., 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 100% of the total amino acids are glycine). Glycine-rich URPs are contemplated for use with the methods and compositions described herein since glycine-rich peptides have an increased conformational freedom (e.g., a characteristic of denatured peptides). The length of a glycine-rich sequence can vary between about 5 amino acids and 400 amino acids. For example, the length of a single, contiguous glycine-rich sequence can contain 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 70, 80, 90, 100, 120, 140, 160, 180, 200, 240, 280, 320 or 400 or more amino acids. A glycine-rich sequence may comprise glycine residues at both ends.

In some embodiments, a URP sequence is optimized to enhance the selectivity of the fusion protein for a particular tissue, cell-type or cell lineage. One can also utilize such URPs to direct the resulting protein to a specific subcellular location: extracellular matrix, nucleus, cytoplasm, cytoskeleton, plasma and/or intracellular membranous structures which include, but are not limited, to coated pits, Golgi apparatus, endoplasmic reticulum, endosome, lysosome, and mitochondria. A variety of these tissue-specific, cell-type specific, subcellular location specific sequences are known and available from numerous protein databases. Such selective URP sequences can be obtained by generating libraries of random or semi-random URP sequences, injecting them into animals or patients, and determining sequences with the desired tissue selectivity in tissue samples. Sequence determination can be performed by mass spectrometry. Using similar methods one can select URP sequences that facilitate oral, buccal, intestinal, nasal, thecal, peritoneal, pulmonary, rectal, or dermal uptake.

In one embodiment, a URP sequence is rich in positively charged amino acids such as arginine or lysine, which favors cellular uptake or transport through membranes. In some embodiments, URP sequences can be designed to contain one or more protease-sensitive sequences. Such URP sequences can be cleaved once the product of the invention has reached its target location. URP sequences can be designed to carry excess negative charges by introducing aspartic acid or glutamic acid residues. Of particular interest are URPs that contain greater than 5%, greater than 6%, 7%, 8%, 9%, 10%, 15%, 30% or more glutamic acid and less than 2% lysine or arginine. Such URPs carry an excess negative charge and as a result have a tendency to adopt open conformations due to electrostatic repulsion between individual negative charges of the peptide. Such an excess negative charge leads to an effective increase in their hydrodynamic radius and as a result it can lead to reduced kidney clearance of such molecules. Thus, one can modulate the effective net charge and hydrodynamic radius of a URP sequence by controlling the frequency and distribution of negatively charged amino acids in the URP sequences.

URPs can include a repetitive amino acid sequence of the format (Motif)x in which a sequence motif forms a direct repeat (ie ABCABCABCABC) or an inverted repeat (ABCCBAABCCBA) and the number of these repeats can be 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 35, 40, 50 or more. URPs (or the repeats inside URPs) often contain only 1, 2, 3, 4, 5 or 6 different types of amino acids. URPs typically consist of repeats of human amino acid sequences that are 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 22, 24, 26, 28, 30, 32, 34, 36 or more amino acids long, but URPs may also consist of non-human amino acid sequences that are 20, 22, 24, 26, 28, 30, 32, 34 36, 38 40, 42, 44, 46, 48, 50 amino acids long.

In one embodiment, URPs are derived from human sequences. The human genome contains many subsequences that are rich in one particular amino acid. Of particular interest are such amino acid sequences that are rich in a hydrophilic amino acid like serine, threonine, glutamate, aspartate, or glycine. Of particular interest are such subsequences that contain few hydrophobic amino acids and are predicted to be unstructured and highly soluable in an aqeuous solution. Such human subsequences can be modified to further improve their utility. Exemplary human sequences for use in designing URPs are shown herein in Tables 24 and 25.

The use of sequences from human proteins is particularly desirable in design of URPs with reduced immunogenicity in a human subject. The URP sequence can be designed to eliminate T cell epitopes to reduce immunogenicity. For instance, one can synthesize a series of semi-random sequences with amino acid compositions that favor denatured, unstructured conformations and evaluate these sequences for the presence of human T cell epitopes and whether they are human sequences. Assays for human T cell epitopes have been described (Stickler, M., et al. (2003) J Immunol Methods, 281: 95-108). One can incorporate human sequences into the design of URP sequences by oligomerizing or concatenating human sequences that have suitable amino acid compositions. These can be direct repeats or inverted repeats or mixtures of different repeats. In one embodiment, the entire URP sequence is from a human sequence.

Non-limiting examples of URPs containing repeating amino acids are: poly-glycine, poly-glutamic acid, poly-aspartic acid, poly-serine, poly-threonine, (GX)n where G is glycine and X is serine, aspartic acid, glutamic acid, threonine, or proline and n is at least 20, (GGX)n where X is serine, aspartic acid, glutamic acid, threonine, or proline and n is at least 13, (GGGX)n where X is serine, aspartic acid, glutamic acid, threonine, or proline and n is at least 10, (GGGGX)n where X is serine, aspartic acid, glutamic acid, threonine, or proline and n is at least 8, (GzX)n where X is serine, aspartic acid, glutamic acid, threonine, or proline, n is at least 15, and z is between 1 and 20.

The number of such repeats can be any number between 10 and 100. Products of the invention may contain URP sequences that are semi-random sequences. Examples are semi-random sequences containing at least 30, 40, 50, 60 or 70% glycine in which the glycines are well dispersed and in which the total concentration of tryptophan, phenylalanine, tyrosine, valine, leucine, and isoleucine is less then 70, 60, 50, 40, 30, 20, or 10% when combined. A preferred semi-random URP sequence contains at least 40% glycine and the total concentration of tryptophan, phenylalanine, tyrosine, valine, leucine, and isoleucine is less then 10%. A more preferred random URP sequence contains at least 50% glycine and the total concentration of tryptophan, phenylalanine, tyrosine, valine, leucine, and isoleucine is less then 5%. URP sequences can be designed by combining the sequences of two or more shorter URP sequences or fragments of URP sequences. Such a combination allows one to better modulate the pharmaceutical properties of the product containing the URP sequences and it allows one to reduce the repetitiveness of the DNA sequences encoding the URP sequences, which can improve expression and reduce recombination of the URP encoding sequences.

A URP sequence can be placed at the N terminus of either the light chain (LC) or heavy chain (HC) of a plasma kallikrein binding protein and a single URP can be attached to either HC or LC at either end. For example, one could combine the VH::CDR3::JH via a linker to VL::JL to make a scFv which could then be fused to a URP.

In one embodiment, a plasma kallikrein binding protein comprises a Fab fragment that inhibits plasma kallikrein and does not bind plasma prekallikrein wherein the LC is fused to a URP of 100 or more (e.g., 120, 140, 160, 180, 200, 300, 400 or more) amino acids and the HC is fused to a URP of 200 or more amino acids (e.g., 220, 240, 260, 280, 300, 350, 400, 450, 500, 600 or more). In one embodiment, the URP is fused to the carboxy terminus of LC and the carboxy terminus of HC. In one embodiment, the URPs have essentially equal amounts of Gly, Ala, Ser, Thr, Glu, and Pro residues. In one embodiment, the URP sequence does not comprise a hexamer repeat. In one embodiment, the plasma kallikrein binding protein (e.g., Fab fragment) is selected from the group consisting of M162-A04, M142-H08, X63-G06, X81-B01, X67-D03, X67-G04, and M160-G12.

In one embodiment, the HC::URP2 and LC::URP1 are produced in a yeast strain such as *Pichia pastoris* (BMC Biotechnol. 2009 Aug. 11; 9:70. PMID 19671134; J Biochem Mol. Biol. 2005 May 31; 38(3):294-9. PMID 15943904; Biotechnol Bioeng. 2006 Jun. 5; 94(2):353-61. PMID 16570317), *Saccharomyces cerevisiae* (BMC Syst Biol. 2010 Oct. 22; 4:141. PMID 20969759; BMC Genomics. 2010 Mar. 26; 11:207. PMID 20346137), or *Hansenula polymorpha* (Appl Microbiol Biotechnol. 2001 July; 56(1-2):157-64. PMID 11499924). One of skill in the art can utilize appropriate promoters and signal sequences for a particular strain of yeast desired for use in producing a fusion protein comprising a plasma kallikrein binding protein and a URP polypeptide.

In one embodiment, the HC::URP2 and LC::URP1 are produced in mammalian cells such as Chinese hamster ovary (CHO) cells. Signal sequences and promoters that are useful for protein production using CHO cells are known in the literature.

Kits

A plasma kallikrein binding protein described herein can be provided in a kit, e.g., as a component of a kit. For example, the kit includes (a) a plasma kallikrein binding protein, e.g., a composition (e.g., a pharmaceutical composition) that includes a plasma kallikrein binding protein, and, optionally (b) informational material. The informational material can be descriptive, instructional, marketing or other material that relates to a method described herein and/or the use of a plasma kallikrein binding protein, e.g., for a method described herein.

The informational material of the kit is not limited in its form. In one embodiment, the informational material can include information about production of the compound, molecular weight of the compound, concentration, date of expiration, batch or production site information, and so forth. In one embodiment, the informational material relates to using the binding protein to treat, prevent, or diagnosis of disorders and conditions, e.g., a plasma kallikrein associated disease or condition.

In one embodiment, the informational material can include instructions to administer a plasma kallikrein binding protein in a suitable manner to perform the methods described herein, e.g., in a suitable dose, dosage form, or mode of administration (e.g., a dose, dosage form, or mode of administration described herein). In another embodiment, the informational material can include instructions to administer a plasma kallikrein binding protein to a suitable subject, e.g., a human, e.g., a human having, or at risk for, a disorder or condition described herein, e.g., a plasma kallikrein associated disease or condition. For example, the material can include instructions to administer a plasma kallikrein binding protein to a patient with a disorder or condition described herein, e.g., a plasma kallikrein associated disease. The informational material of the kits is not limited in its form. In many cases, the informational material, e.g., instructions, is provided in print but may also be in other formats, such as computer readable material.

A plasma kallikrein binding protein can be provided in any form, e.g., liquid, dried or lyophilized form. It is preferred that a plasma kallikrein binding protein be substantially pure and/or sterile. When a plasma kallikrein binding protein is provided in a liquid solution, the liquid solution preferably is an aqueous solution, with a sterile aqueous solution being preferred. When a plasma kallikrein binding protein is provided as a dried form, reconstitution generally is by the addition of a suitable solvent. The solvent, e.g., sterile water or buffer, can optionally be provided in the kit.

The kit can include one or more containers for the composition containing a plasma kallikrein binding protein. In some embodiments, the kit contains separate containers, dividers or compartments for the composition and informational material. For example, the composition can be contained in a bottle, vial, or syringe, and the informational material can be contained in association with the container. In other embodiments, the separate elements of the kit are contained within a single, undivided container. For example, the composition is contained in a bottle, vial or syringe that has attached thereto the informational material in the form of a label. In some embodiments, the kit includes a plurality (e.g., a pack) of individual containers, each containing one or more unit dosage forms (e.g., a dosage form described herein) of a plasma kallikrein binding protein. For example, the kit includes a plurality of syringes, ampules, foil packets, or blister packs, each containing a single unit dose of a plasma kallikrein binding protein. The containers of the kits can be air tight, waterproof (e.g., impermeable to changes in moisture or evaporation), and/or light-tight.

The kit optionally includes a device suitable for administration of the composition, e.g., a syringe, inhalant, dropper (e.g., eye dropper), swab (e.g., a cotton swab or wooden swab), or any such delivery device. In one embodiment, the device is an implantable device that dispenses metered doses of the binding protein. The disclosure also features a method of providing a kit, e.g., by combining components described herein.

Treatments

Proteins that bind to plasma kallikrein, e.g., as described herein, have therapeutic and prophylactic utilities, particularly in human subjects. These binding proteins are administered to a subject to treat, prevent, and/or diagnose a variety of disorders and conditions, including e.g., a plasma kallikrein associated disease, or even to cells in culture, e.g., in vitro or ex vivo. For example, these binding proteins can be used to modify the effects of plasma kallikrein released from cells in culture (Lilla et al., J Biol Chem. 284(20):13792-13803 (2009)). Treating includes administering an amount effective to alleviate, relieve, alter, remedy, ameliorate, improve or affect the disorder, the symptoms of the disorder or the predisposition toward the disorder. The treatment may also delay onset, e.g., prevent onset, or prevent deterioration of a disease or condition.

As used herein, an amount of a target-binding agent effective to prevent a disorder, or a prophylactically effective amount of the binding agent refers to an amount of a target binding agent, e.g., an plasma kallikrein binding protein, e.g., an anti-plasma kallikrein antibody described herein, which is effective, upon single- or multiple-dose administration to the subject, for preventing or delaying the occurrence of the onset or recurrence of a disorder, e.g., a disorder described herein, e.g., a plasma kallikrein associated disease.

Methods of administering plasma kallikrein binding proteins and other agents are also described in "Pharmaceutical Compositions." Suitable dosages of the molecules used can depend on the age and weight of the subject and the particular drug used. The binding proteins can be used as competitive agents to inhibit, reduce an undesirable interaction, e.g., between plasma kallikrein and its substrate (e.g., Factor XII or HMWK). The dose of the plasma kallikrein binding protein can be the amount sufficient to block 90%, 95%, 99%, or 99.9% of the activity of plasma kallikrein in the patient, especially at the site of disease. Depending on the disease, this may require 0.1, 1.0, 3.0, 6.0, or 10.0 mg/Kg. For an IgG having a molecular mass of 150,000 g/mole (two binding sites), these doses correspond to approximately 18 nM, 180 nM, 540 nM, 1.08 and 1.8 μM of binding sites for a 5 L blood volume.

In one embodiment, the plasma kallikrein binding proteins are used to inhibit an activity (e.g., inhibit at least one activity of plasma kallikrein, e.g., reduce Factor XIIa and/or bradykinin production) of plasma kallikrein, e.g., in vivo. The binding proteins can be used by themselves or conjugated to an agent, e.g., a cytotoxic drug, cytotoxin enzyme, or radioisotope. This method includes: administering the binding protein alone or attached to an agent (e.g., a cytotoxic drug), to a subject requiring such treatment. For example, plasma kallikrein binding proteins that do not substantially inhibit plasma kallikrein may be used to deliver nanoparticles containing agents, such as toxins, to plasma kallikrein associated cells or tissues, e.g., to treat a plasma kallikrein-associate disorder.

Because the plasma kallikrein binding proteins recognize plasma kallikrein expressing cells and can bind to cells that are associated with (e.g., in proximity of or intermingled with) a plasma kallikrein associated disorder or condition, plasma kallikrein binding proteins can be used to inhibit an activity (e.g., inhibit at least one activity of plasma kallikrein, e.g., reduce Factor XIIa and/or bradykinin production) any such cells and inhibit the plasma kallikrein associated disease. Reducing plasma kallikrein activity can indirectly inhibit cells which may be dependent on the plasma kallikrein activity for the development and/or progression of a plasma kallikrein-associated disorder.

The binding proteins may be used to deliver an agent (e.g., any of a variety of cytotoxic and therapeutic drugs) to cells and tissues where plasma kallikrein is present. Exemplary agents include a compound emitting radiation, molecules of plants, fungal, or bacterial origin, biological proteins, and mixtures thereof. The cytotoxic drugs can be intracellularly acting cytotoxic drugs, such as toxins short range radiation emitters, e.g., short range, high energy α-emitters.

To target plasma kallikrein expressing cells, a prodrug system can be used. For example, a first binding protein is conjugated with a prodrug which is activated only when in close proximity with a prodrug activator. The prodrug activator is conjugated with a second binding protein, preferably one which binds to a non competing site on the target molecule. Whether two binding proteins bind to competing or non competing binding sites can be determined by conventional competitive binding assays. Exemplary drug prodrug pairs are described in Blakely et al., (1996) Cancer Research, 56:3287 3292.

The plasma kallikrein binding proteins can be used directly in vivo to eliminate antigen-expressing cells via natural complement-dependent cytotoxicity (CDC) or antibody dependent cellular cytotoxicity (ADCC). The binding proteins described herein can include complement binding effector domain, such as the Fc portions from IgG1, -2, or -3 or corresponding portions of IgM which bind complement. In one embodiment, a population of target cells is ex vivo treated with a binding agent described herein and appropriate effector cells. The treatment can be supplemented by the addition of complement or serum containing complement. Further, phagocytosis of target cells coated with a binding protein described herein can be improved by binding of complement proteins. In another embodiment target, cells coated with the binding protein which includes a complement binding effector domain are lysed by complement.

Methods of administering plasma kallikrein binding proteins are described in "Pharmaceutical Compositions." Suitable dosages of the molecules used will depend on the age and weight of the subject and the particular drug used. The binding proteins can be used as competitive agents to inhibit or reduce an undesirable interaction, e.g., between a natural or pathological agent and the plasma kallikrein.

The plasma kallikrein binding protein can be used to deliver macro and micromolecules, e.g., a gene into the cell for gene therapy purposes into the endothelium or epithelium and target only those tissues expressing the plasma kallikrein. The binding proteins may be used to deliver a variety of cytotoxic drugs including therapeutic drugs, a compound emitting radiation, molecules of plants, fungal, or bacterial origin, biological proteins, and mixtures thereof. The cytotoxic drugs can be intracellularly acting cytotoxic drugs, such as short range radiation emitters, including, for example, short range, high energy α emitters, as described herein.

In the case of polypeptide toxins, recombinant nucleic acid techniques can be used to construct a nucleic acid that encodes the binding protein (e.g., antibody or antigen-binding fragment thereof) and the cytotoxin (or a polypeptide component thereof) as translational fusions. The recombinant nucleic acid is then expressed, e.g., in cells and the encoded fusion polypeptide isolated.

Alternatively, the plasma kallikrein binding protein can be coupled to high energy radiation emitters, for example, a radioisotope, such as ^{131}I, a γ-emitter, which, when localized at a site, results in a killing of several cell diameters. See, e.g., S. E. Order, "Analysis, Results, and Future Prospective of the Therapeutic Use of Radiolabeled Antibody in Cancer Therapy", *Monoclonal Antibodies for Cancer Detection and Therapy*, R. W. Baldwin et al. (eds.), pp 303 316 (Academic Press 1985). Other suitable radioisotopes include a emitters, such as ^{212}Bi, ^{213}Bi, and ^{211}At, and b emitters, such as ^{186}Re and ^{90}Y. Moreover, ^{177}Lu may also be used as both an imaging and cytotoxic agent.

Radioimmunotherapy (RIT) using antibodies labeled with ^{131}I, ^{90}Y and ^{177}Lu is under intense clinical investigation. There are significant differences in the physical characteristics of these three nuclides and as a result, the choice of radionuclide is very critical in order to deliver maximum radiation dose to a tissue of interest. The higher beta energy particles of ^{90}Y may be good for bulky tumors. The relatively low energy beta particles of ^{131}I are ideal, but in vivo dehalogenation of radioiodinated molecules is a major disadvantage for internalizing antibody. In contrast, ^{177}Lu has low energy beta particle with only 0.2-0.3 mm range and delivers much lower radiation dose to bone marrow compared to ^{90}Y. In addition, due to longer physical half-life (compared to ^{90}Y), the residence times are higher. As a result, higher activities (more mCi amounts) of ^{177}Lu labeled agents can be administered with comparatively less radiation dose to marrow. There have been several clinical studies investigating the use of ^{177}Lu labeled antibodies in the treatment of various cancers. (Mulligan T et al., 1995, *Clin. Canc. Res.* 1: 1447-1454; Meredith R F, et al., 1996, *J. Nucl. Med.* 37:1491-1496; Alvarez R D, et al., 1997, *Gynecol. Oncol.* 65: 94-101).

Exemplary Diseases and Conditions

A plasma kallikrein binding protein described herein is useful to treat (or prevent) a disease or condition in which plasma kallikrein activity is implicated, e.g., a disease or condition described herein, or to treat (or prevent) one or more symptoms associated therewith. In some embodiments, the plasma kallikrein binding protein (e.g., plasma kallikrein binding IgG or Fab) inhibits plasma kallikrein activity.

Examples of such diseases and conditions which can be treated (or prevented) by a plasma kallikrein binding protein described herein include: rheumatoid arthritis, gout, intestinal bowel disease, oral mucositis, neuropathic pain, inflammatory pain, spinal stenosis-degenerative spine disease, arterial or venous thrombosis, post operative ileus, aortic aneurysm, osteoarthritis, vasculitis, edema, hereditary angioedema, cerebral edema, pulmonary embolism, stroke, clotting induced by ventricular assistance devices or stents, head trauma or peri-tumor brain edema, sepsis, acute middle cerebral artery (MCA) ischemic event (stroke), restenosis (e.g., after angioplasty), systemic lupus erythematosis nephritis, and burn injury. A plasma kallikrein binding protein described herein can also be used to promote wound healing. A plasma kallikrein binding protein described herein can also be used as an oncology treatment by mechanisms that include, but are not limited to, blocking production of pro-angiogenic bradykinin.

A therapeutically effective amount of a plasma kallikrein binding protein can be administered to a subject having or suspected of having a disorder in which plasma kallikrein activity is implicated, thereby treating (e.g., ameliorating or improving a symptom or feature of a disorder, slowing, stabilizing and/or halting disease progression) the disorder.

The plasma kallikrein binding protein can be administered in a therapeutically effective amount. A therapeutically effective amount of a plasma kallikrein binding protein is the amount which is effective, upon single or multiple dose administration to a subject, in treating a subject, e.g., curing, alleviating, relieving or improving at least one symptom of a disorder in a subject to a degree beyond that expected in the absence of such treatment. A therapeutically effective amount of the composition may vary according to factors such as the disease state, age, sex, and weight of the individual, and the ability of the compound to elicit a desired response in the individual. A therapeutically effective amount is also one in which any toxic or detrimental effects of the composition are outweighed by the therapeutically beneficial effects. A therapeutically effective dosage preferably modulates a measurable parameter, favorably, relative to untreated subjects. The ability of a compound to affect (e.g., inhibit) a measurable parameter can be evaluated in an animal model system predictive of efficacy in a human disorder.

Dosage regimens can be adjusted to provide the optimum desired response (e.g., a therapeutic response). For example, a single bolus may be administered, several divided doses may be administered over time or the dose may be proportionally reduced or increased as indicated by the exigencies of the therapeutic situation. It is especially advantageous to formulate parenteral compositions in dosage unit form for ease of administration and uniformity of dosage. Dosage unit form as used herein refers to physically discrete units suited as unitary dosages for the subjects to be treated; each unit contains a predetermined quantity of active compound calculated to produce the desired therapeutic effect in association with the required pharmaceutical carrier.

Rheumatoid Arthritis

Rheumatoid arthritis (RA) is an autoimmune, chronic inflammatory disease that causes joint swelling and pain and normally results in joint destruction. RA generally follows a relapsing/remitting course, with "flares" of disease activity interspersed with remissions of disease symptoms. RA is associated with a number of additional inflammatory disorders, including Sjogren's syndrome (dry eyes and mouth caused by inflammation of tear and saliva glands), pleuritis (inflammation of the pleura that causes pain upon deep breath and coughing), rheumatoid nodules (nodular sites of inflammation that develop within the lungs), pericarditis (inflammation of the pericardium that causes pain when lying down or leaning forward), Felty syndrome (splenomegaly and leucopenia observed in conjunction with RA, making the subject prone to infection), and vasculitis (an inflammation of the blood vessels which can block blood flow). Plasma kallikrein has been implicated in rheumatoid arthritis.

Symptoms of active RA include fatigue, lack of appetite, low grade fever, muscle and joint aches, and stiffness. Muscle and joint stiffness are usually most notable in the morning and after periods of inactivity. During flares, joints frequently become red, swollen, painful, and tender, generally as a consequence of synovitis.

Treatment for rheumatoid arthritis involves a combination of medications, rest, joint strengthening exercises, and joint protection. Two classes of medications are used in treating rheumatoid arthritis: anti-inflammatory "first-line drugs," and "Disease-Modifying Antirheumatic Drugs" (DMARDs). The first-line drugs include NSAIDS (e.g., aspirin, naproxen, ibuprofen, and etodolac) and cortisone (corticosteroids). DMARDs, such as gold (e.g., gold salts, gold thioglucose, gold thiomalate, oral gold), methotrexate, sulfasalazine, D-penicillamine, azathioprine, cyclophosphamide, chlorambucil, and cyclosporine, leflunomide, etanercept, infliximab, anakinra, and adalimumab, and hydroxychloroquine, promote disease remission and prevent progressive joint destruction, but they are not anti-inflammatory agents.

The disclosure provides methods of treating (e.g., ameliorating, stabilizing, or eliminating one or more symptoms or ameliorating or stabilizing the subject's score on a RA scale) rheumatoid arthritis by administering a plasma kallikrein binding protein (e.g., a therapeutically effective amount of a plasma kallikrein binding protein) to a subject having or suspected of having RA. Additionally provided are methods of treating RA by administering a plasma kallikrein binding protein (e.g., a therapeutically effective amount of a plasma kallikrein binding protein) in combination with a second therapy, e.g., with at least one anti-inflammatory "first line drug" (e.g., an NSAID and/or cortisone) and/or a DMARD. The disclosure also provides methods of preventing rheumatoid arthritis or a symptom thereof by administering a plasma kallikrein binding protein (e.g., a prophylactically effective amount of a plasma kallikrein binding protein) to a subject at risk of developing RA (e.g., a subject having a family member with RA or a genetic predisposition thereto).

Further provided are methods of treating (e.g., ameliorating, stabilizing, or eliminating one or more symptoms) rheumatoid arthritis associated disorders (Sjogren's syndrome, pleuritis, pulmonary rheumatoid nodules, pericarditis, Felty syndrome, and vasculitis) by administering a plasma kallikrein binding protein (e.g., a therapeutically effective amount of a plasma kallikrein binding protein) to a subject having or suspected of having RA.

Scales useful for assessing RA and symptoms of RA include, e.g., the Rheumatoid Arthritis Severity Scale (RASS; Bardwell et al., (2002) *Rheumatology* 41(1):38-45), SF-36 Arthritis Specific Health Index (ASHI; Ware et al., (1999) *Med. Care.* 37(5 Suppl):MS40-50), Arthritis Impact Measurement Scales or Arthritis Impact Measurement Scales 2 (AIMS or AIMS2; Meenan et al. (1992) *Arthritis Rheum.* 35(1):1-10); the Stanford Health Assessment Questionnaire (HAQ), HAQII, or modified HAQ (see, e.g., Pincus et al. (1983) *Arthritis Rheum.* 26(11):1346-53).

Guidance for the determination of the dosage that delivers a therapeutically effective amount of a plasma kallikrein binding protein may be obtained from animal models of rheumatoid arthritis, such as collagen-induced arthritis (CIA), which is induced, typically in rodents, by immunization with autologous or heterologous type II collagen in adjuvant (Williams et al. Methods Mol Med. 98:207-16 (2004)).

Gout

Gout is a condition that results from crystals of uric acid depositing in tissues of the body. Gout is characterized by an overload of uric acid in the body and recurring attacks of joint inflammation (arthritis). Chronic gout can lead to deposits of hard lumps of uric acid in and around the joints, decreased kidney function, and kidney stones. Gout is often related to an inherited abnormality in the body's ability to process uric acid. Uric acid is a breakdown product of purines, which are part of many foods. An abnormality in handling uric acid can cause attacks of painful arthritis (gout attack), kidney stones, and blockage of the kidney filtering tubules with uric acid crystals, leading to kidney failure. Some patients may only develop elevated blood uric acid levels (hyperuricemia) without having arthritis or kidney problems.

Symptoms of gout include, e.g., excruciating and unexpected pain, swelling, redness, warmth and stiffness in the affected foot or other parts of the body, and low-grade fever.

Treatments for gout include, e.g., nonsteroidal anti-inflammatory drugs (NSAIDs), colchicine and oral glucocorticoids, intra-articular glucocorticoids administered via a joint injection, xanthine oxidase inhibitors (e.g., allopurinol, febuxostat), uricosurics (e.g., probenecid, EDTA), urate oxidases (e.g., pegloticase), sodium bicarbonate, and low purine diet.

The disclosure provides methods of treating (e.g., ameliorating, stabilizing, or eliminating one or more symptoms or the worsening of) gout by administering a plasma kallikrein binding protein (e.g., a therapeutically effective amount of a plasma kallikrein binding protein) to a subject having or suspected of having gout. Additionally provided are methods of treating gout by administering a plasma kallikrein binding protein (e.g., a therapeutically effective amount of a kallikrein binding protein) in combination with a second therapy, e.g., an NSAID, a colchicine, an oral glucocorticoid, an intra-articular glucocorticoid administered via a joint injection, a xanthine oxidase inhibitor (e.g., allopurinol, febuxostat), a uricosuric (e.g., probenecid, EDTA), a urate oxidase (e.g., pegloticase), sodium bicarbonate, and/or low purine diet. The disclosure also provides methods of preventing gout or a symptom thereof by administering a plasma kallikrein binding protein (e.g., a prophylactically effective amount of a plasma kallikrein binding protein) to a subject at risk of developing gout (e.g., a subject having a family member with gout or a genetic predisposition thereto).

Guidance for the determination of the dosage that delivers a therapeutically effective amount of a plasma kallikrein binding protein may be obtained from animal models of gout, see, e.g., Reginato and Olsen, Curr Opin Rheumatol. 19(2): 134-45 (2007) and references cited therein.

Intestinal Bowel Disease (IBD)

Inflammatory bowel disease (IBD) is a group of inflammatory conditions of the large intestine and, in some cases, the small intestine. The main forms of IBD are Crohn's disease and ulcerative colitis (UC). Accounting for far fewer cases are other forms of IBD: collagenous colitis, lymphocytic colitis, ischaemic colitis, diversion colitis, Behçet's syndrome, infective colitis, and indeterminate colitis. The main difference between Crohn's disease and UC is the location and nature of the inflammatory changes. Crohn's can affect any part of the gastrointestinal tract, from mouth to anus (skip lesions), although a majority of the cases start in the terminal ileum Ulcerative colitis, in contrast, is restricted to the colon and the rectum Microscopically, ulcerative colitis is restricted to the mucosa (epithelial lining of the gut), while Crohn's disease affects the whole bowel wall. Finally, Crohn's disease and ulcerative colitis present with extra-intestinal manifestations (such as liver problems, arthritis, skin manifestations and eye problems) in different proportions.

Symptoms of IBD include abdominal pain, vomiting, diarrhea, hematochezia, weight loss, weight gain and various associated complaints or diseases (arthritis, pyoderma gangrenosum, primary sclerosing cholangitis). Diagnosis is generally by colonoscopy with biopsy of pathological lesions. Rarely, a definitive diagnosis of neither Crohn's disease nor ulcerative colitis can be made because of idiosyncrases in the presentation. In this case, a diagnosis of indeterminate colitis may be made.

Treatment for IBD, depending on the level of severity, may require immunosuppression to control the symptoms. Immunosuppresives such as azathioprine, methotrexate, or 6-mercaptopurine can be used. More commonly, treatment of IBD requires a form of mesalamine Often, steroids are used to control disease flares and were once acceptable as a maintenance drug. Biologicals, such as infliximab, have been used to treat patients with Crohn's disease or Ulcerative Colitis. Severe cases may require surgery, such as bowel resection, strictureplasty or a temporary or permanent colostomy or ileostomy. Alternative medicine treatments for IBD exist in various forms however such methods concentrate on controlling underlying pathology in order to avoid prolonged steroidal exposure or surgical excision. Usually the treatment is started by administering drugs, such as prednisone, with high anti-inflammatory affects. Once the inflammation is successfully controlled, the patient is usually switched to a lighter drug, such as asacol—a mesalamine—to keep the disease in remission. If unsuccessful, a combination of the aforementioned immunosuppressant drugs with a mesalamine (which may also have an anti-inflammatory effect) may or may not be administered, depending on the patient.

The disclosure provides methods of treating (e.g., ameliorating, stabilizing, or eliminating one or more symptoms of) IBD by administering a plasma kallikrein binding protein (e.g., a therapeutically effective amount of a plasma kallikrein binding protein) to a subject having or suspected of having IBD. Additionally provided are methods of treating IBD by administering a plasma kallikrein binding protein (e.g., a therapeutically effective amount of a kallikrein binding protein) in combination with a second therapy, e.g., an immunosuppressive (e.g., azathioprine, methotrexate, 6-mercaptopurine), a mesalamine, a steroid, and/or infliximab. The disclosure also provides methods of preventing IBD or a symptom thereof by administering a plasma kallikrein binding protein (e.g., a prophylactically effective amount of a plasma kallikrein binding protein) to a subject at risk of developing IBD (e.g., a subject having a family member with IBD or a genetic predisposition thereto).

Guidance for the determination of the dosage that delivers a therapeutically effective amount of a plasma kallikrein binding protein may be obtained from animal models of IBD, see, e.g., those described in U.S. Pat. No. 6,114,382, WO 2004/071186, and references cited therein.

Oral Mucositis

Oral mucositis is the painful inflammation and ulceration of the mucous membranes in the mouth, usually as an adverse effect of chemotherapy and radiotherapy treatment for cancer.

Symptoms of oral mucositis include, e.g., ulcers, peripheral erythema, burning sensation accompanied by reddening, trouble speaking, eating, or even opening the mouth, and dyseusia (alteration in taste perception).

Treatment for oral mucositis includes oral hygiene (salt mouthwash, GELCLAIR®, CAPHOSOL®, MUGARD®), palifermin (a human keratinocyte growth factor), cytokines and other modifiers of inflammation (e.g., IL-1, IL-11, TGF-beta3), amino acid supplementation (e.g., glutamine), vitamins, colony-stimulating factors, cryotherapy, and laser therapy.

The disclosure provides methods of treating (e.g., ameliorating, reducing, or eliminating one or more symptoms, or stabilizing the subject's score on a mucositis scale) oral mucositis by administering a plasma kallikrein binding protein (e.g., a therapeutically effective amount of a plasma kallikrein binding protein) to a subject having or suspected of having oral mucositis. Additionally provided are methods of treating oral mucositis by administering a plasma kallikrein binding protein (e.g., a therapeutically effective amount of a plasma kallikrein binding protein) in combination with a second therapy, e.g., oral hygiene (salt mouthwash, GELCLAIR®, CAPHOSOL®, MUGARD®), palifermin (a human keratinocyte growth factor), a cytokine and/or a modifier of inflammation (e.g., IL-1, IL-11, TGF-beta3), an amino acid supplementation (e.g., glutamine), a vitamin, a colony-stimulating factor, cryotherapy, and/or laser therapy. The disclosure also provides methods of preventing oral mucositis or a symptom thereof by administering a plasma kallikrein binding protein (e.g., a prophylactically effective amount of a plasma kallikrein binding protein) to a subject at risk of developing oral mucositis (e.g., a subject that has undergone or is undergoing chemotherapy or radiotherapy).

Scales useful for assessing oral mucositis include the World Health Organization (WHO) Oral Toxicity score (Handbook for reporting results of cancer treatment. Geneva, Switzerland: World Health Organization; 1979:15-22), National Cancer Institute Common Toxicity Criteria (NCI-CTC) for Oral Mucositis (National Cancer Institute Common Toxicity Criteria. Version 2.0, Jun. 1, 1999, Sonis et al., Cancer. 85:2103-2113 (1999)), and Oral Mucositis Assessment Scale (OMAS).

Guidance for the determination of the dosage that delivers a therapeutically effective amount of a plasma kallikrein binding protein may be obtained from animal models of oral mucositis, such as an animal model of oral mucositis induced by conditioning regimen of haematopoietic stem cell transplantation (Chen et al., Zhonghua Kou Qiang Yi Xue Za Zhi. 42(11):672-6 (2007)).

Neuropathic Pain

Neuropathic pain is a complex, chronic pain state that usually is accompanied by tissue injury. With neuropathic pain, the nerve fibers themselves may be damaged, dysfunctional or injured. These damaged nerve fibers send incorrect signals to other pain centers. The impact of nerve fiber injury includes a change in nerve function both at the site of injury and areas around the injury.

Symptoms of neuropathic pain include, e.g., shooting and burning pain and tingling and numbness.

Treatments for neuropathic pain include, e.g., medications (e.g., non-steroidal anti-inflammatory drugs (NSAIDs) (e.g., ALEVE®, MOTRIN®, or morphine), anticonvulsant, and antidepressant drugs), and invasive or implantable devices (e.g., electrical stimulation).

The disclosure provides methods of treating (e.g., ameliorating, reducing, or eliminating one or more symptoms of or stabilizing the subject's score on a pain scale) neuropathic pain by administering a plasma kallikrein binding protein (e.g., a therapeutically effective amount of a plasma kallikrein binding protein) to a subject having or suspected of having neuropathic pain. Additionally provided are methods of treating neuropathic pain by administering a plasma kallikrein binding protein (e.g., a therapeutically effective amount of a plasma kallikrein binding protein) in combination with a second therapy, e.g., a nonsurgical treatment ((e.g., a non-steroidal anti-inflammatory drug (NSAID) (e.g., ALEVE®, MOTRIN®, or morphine), an anticonvulsant, and/or an antidepressant drug), and/or an invasive or implantable device (e.g., electrical stimulation). The disclosure also provides methods of preventing neuropathic pain or a symptom thereof by administering a plasma kallikrein binding protein (e.g., a prophylactically effective amount of a plasma kallikrein binding protein) to a subject at risk of developing neuropathic pain (e.g., a subject that has experienced tissue injury).

Scales useful for the assessment of neuropathic pain include, e.g., Wong-Baker FACES Pain Rating Scale (Wong-Baker FACES Pain Rating Scale Foundation), Visual analog scale (VAS) (Huskisson, J. Rheumatol. 9 (5): 768-9 (1982)), McGill Pain Questionnaire (MPQ) (Melzack, Pain 1 (3): 277-99 (1975)), Descriptor differential scale (DDS) (Gracety and Kwilosz, Pain 35 (3): 279-88 (1988)), Faces Pain Scale-Revised (FPS-R) (Hicks et al., Pain 93 (2): 173-83 (2001)), Numerical 11 point box (BS-11) (Jensen et al., Clin J Pain 5 (2): 153-9 (1989)), Numeric Rating Scale (NRS-11) (Hartrick et al., Pain Pract 3 (4): 310-6 (2003)), Dolorimeter Pain Index (DPI) (Hardy et al., (1952). Pain Sensations and Reactions. Baltimore: The Williams & Wilkins Co.), and Brief Pain Inventory (BPI) (Cleeland and Ryan *Ann. Acad. Med. Singap.* 23 (2): 129-38 (1994)).

Guidance for the determination of the dosage that delivers a therapeutically effective amount of a plasma kallikrein binding protein may be obtained from animal models of neuropathic pain, see, e.g., those described in Martin et al., Methods Mol. Med. 84:233-42 (2003) and references cited therein.

Inflammatory Pain

Inflammatory pain is caused by an insult such as penetration wounds, burns, extreme cold, fractures, arthritis, autoimmune conditions, excessive stretching, infections and vasoconstriction to the integrity of tissues at a cellular level. During inflammation a complex neuro-immune interaction results in primary hyperalgesia, in which a large range of inflammatory molecules including prostaglandins and bradykinin induce and maintain the altered nociceptor sensitivity.

Treatments for inflammatory pain include, e.g., non-steroidal anti-inflammatory drugs (NSAIDs) and corticosteroids.

The disclosure provides methods of treating (e.g., ameliorating, reducing, or eliminating one or more symptoms of) inflammatory pain by administering a plasma kallikrein binding protein (e.g., a therapeutically effective amount of a plasma kallikrein binding protein) to a subject having or suspected of having inflammatory pain. Additionally provided are methods of treating inflammatory pain by administering a plasma kallikrein binding protein (e.g., a therapeutically effective amount of a plasma kallikrein binding protein) in combination with a second therapy, e.g., a non-steroidal anti-inflammatory drug (NSAID) and/or a corticosteroid. The disclosure also provides methods of preventing inflammatory pain or a symptom thereof by administering a plasma kallikrein binding protein (e.g., a prophylactically effective amount of a plasma kallikrein binding protein) to a subject at risk of developing inflammatory pain (e.g., a subject that has experienced an insult, e.g., such as a penetration wound, a burn, extreme cold, a fracture, arthritis, an autoimmune condition, excessive stretching, or infection).

Scales useful for the assessment of inflammatory pain include, e.g., Wong-Baker FACES Pain Rating Scale (Wong-Baker FACES Pain Rating Scale Foundation), Visual analog scale (VAS) (Huskisson, J. Rheumatol. 9 (5): 768-9 (1982)), McGill Pain Questionnaire (MPQ) (Melzack, Pain 1 (3): 277-99 (1975)), Descriptor differential scale (DDS) (Gracety and Kwilosz, Pain 35 (3): 279-88 (1988)), Faces Pain Scale-Revised (FPS-R) (Hicks et al., Pain 93 (2): 173-83 (2001)), Numerical 11 point box (BS-11) (Jensen et al., Clin J Pain 5 (2): 153-9 (1989)), Numeric Rating Scale (NRS-11) (Hartrick et al., Pain Pract 3 (4): 310-6 (2003)), Dolorimeter Pain Index (DPI) (Hardy et al., (1952). Pain Sensations and Reactions. Baltimore: The Williams & Wilkins Co.), and Brief Pain Inventory (BPI) (Cleeland and Ryan *Ann. Acad. Med. Singap.* 23 (2): 129-38 (1994)).

Guidance for the determination of the dosage that delivers a therapeutically effective amount of a plasma kallikrein binding protein may be obtained from animal models of inflammatory pain such as an animal model of chronic inflammatory pain (Wilson et al., Eur J Pain. 10(6):537-49 (2006)) and an inflammatory model of pain and hyperalgesia (Ren and Dubner, ILAR J. 40(3):111-118 (1999)).

Spinal Stenosis

Spinal stenosis is a medical condition in which the spinal canal narrows and compresses the spinal cord and nerves. This is usually due to the common occurrence of spinal degeneration that occurs with aging. It can also sometimes be caused by spinal disc herniation, osteoporosis or a tumor. Spinal stenosis may affect the cervical, thoracic or lumbar spine. In some cases, it may be present in all three places in the same patient.

Symptoms of spinal stenosis include, e.g., pain or cramping in the legs, radiating back and hip pain, pain in the neck and shoulders, loss of balance, and loss of bowel or bladder function (cauda equina syndrome).

Treatments for spinal stenosis include, e.g., nonsurgical treatments (e.g., physical therapy, non-steroidal anti-inflammatory drugs (NSAIDs) (e.g., aspirin, ibuprofen and indomethacin), analgesics (e.g., acetaminophen), chondroitin sulfate, glucosamine, rest or restricted activity, back brace or corset, epidural steroid injections (e.g., corticosteroid)), and surgery (e.g., decompressive laminectomy, laminotomy and fusion).

The disclosure provides methods of treating (e.g., ameliorating, reducing, or eliminating one or more symptoms of) spinal stenosis by administering a plasma kallikrein binding protein (e.g., a therapeutically effective amount of a plasma kallikrein binding protein) to a subject having or suspected of having spinal stenosis. Additionally provided are methods of treating spinal stenosis by administering a plasma kallikrein binding protein (e.g., a therapeutically effective amount of a plasma kallikrein binding protein) in combination with a second therapy, e.g., a nonsurgical treatment (e.g., physical therapy and/or a nonsteroidal anti-inflammatory drug (NSAID) (e.g., aspirin, ibuprofen or indomethacin), an analgesic (e.g., acetaminophen), chondroitin sulfate, glucosamine, rest or restricted activity, a back brace or corset, an epidural steroid injection (e.g., corticosteroid), and/or surgery (e.g., decompressive laminectomy, laminotomy and/or fusion). The disclosure also provides methods of preventing spinal stenosis or a symptom thereof by administering a plasma kallikrein binding protein (e.g., a prophylactically effective amount of a plasma kallikrein binding protein) to a subject at risk of developing spinal stenosis (e.g., a subject that has spinal degeneration).

Guidance for the determination of the dosage that delivers a therapeutically effective amount of a plasma kallikrein binding protein may be obtained from animal models of spinal stenosis, such as a model of lumbar spinal stenosis (Sekiguchi et al., Spine 29, 1105-1111 (2004)).

Arterial and Venous Thrombosis

Arterial thrombosis is the formation of a thrombus within an artery. In most cases, arterial thrombosis follows rupture of atheroma, and is therefore referred to as atherothrombosis.

Arterial thrombosis is associated with a number of disorders, including stroke and myocardial infarction. In thrombotic stroke, a thrombus (blood clot) usually forms around atherosclerotic plaques. Since blockage of the artery is gradual, onset of symptomatic thrombotic strokes is slower. Thrombotic stroke can be divided into two categories—large vessel disease and small vessel disease. The former affects vessels such as the internal carotids, vertebral and the circle of Willis. The latter can affect smaller vessels such as the branches of the circle of Willis Myocardial infarction (MI) is caused by an infarct (death of tissue due to ischemia), often due to the obstruction of the coronary artery by a thrombus. MI can quickly become fatal if emergency medical treatment is not received promptly.

Venous thrombosis is a blood clot that forms within a vein. If a piece of a blood clot formed in a vein breaks off, it can be transported to the right side of the heart, and from there into the lungs. A piece of thrombus that is transported in this way is an embolism and the process of forming a thrombus that becomes embolic is called a thromboembolism. An embolism that lodges in the lungs is a pulmonary embolism (PE). A pulmonary embolus is a very serious condition that can be fatal if not recognized and treated promptly.

Superficial venous thromboses can cause discomfort but generally do not cause serious consequences, unlike the deep venous thromboses (DVTs) that form in the deep veins of the legs or in the pelvic veins. Systemic embolisms of venous origin can occur in patients with an atrial or ventricular septal defect, through which an embolus may pass into the arterial system. Such an event is termed a paradoxical embolism.

Prevention of arterial and/or venous thrombosis includes medications (e.g., anticoagulants (e.g., heparin), aspirin, and vitamin E) and mechanical methods (e.g., mechanical leg pumps (pneumatic compression stockings)).

The disclosure provides methods of treating (e.g., ameliorating, reducing, or eliminating one or more symptoms of) arterial and/or venous thrombosis by administering a plasma kallikrein binding protein (e.g., a therapeutically effective amount of a plasma kallikrein binding protein) to a subject having or suspected of having arterial and/or venous thrombosis. Additionally provided are methods of treating arterial and/or venous thrombosis by administering a plasma kallikrein binding protein (e.g., a therapeutically effective amount of a plasma kallikrein binding protein) in combination with a second therapy, e.g., an anticoagulant (e.g., heparin), aspirin, and/or vitamin E and/or a mechanical method (e.g., a mechanical leg pump (pneumatic compression stockings). The disclosure also provides methods of preventing arterial and/or venous thrombosis or a symptom thereof by administering a plasma kallikrein binding protein (e.g., a prophylactically effective amount of a plasma kallikrein binding protein) to a subject at risk of developing arterial and/or venous thrombosis (e.g., a subject that has experienced a stroke or myocardial infarction).

Guidance for the determination of the dosage that delivers a therapeutically effective amount of a plasma kallikrein binding protein may be obtained from animal models of arterial or venous thrombosis, such as a double-tuck model of arterial thrombosis (Gomez-Jorge et al., J. Vasc. Inter. Rad. 9(4): 633-638 (1998), a model of venous thrombosis in rat with low flow conditions in the venous blood stream (Fredrich et al., Blood Coagul Fibrinolysis. 5(2):243-8 (1994)), and a canine model for venous thrombosis and spontaneous pulmonary embolism (Frisbiel, Spinal Cord 43, 635-639 (2005)).

Postoperative Ileus

Postoperative ileus is a temporary paralysis of a portion of the intestines typically after an abdominal surgery. Postoperative ileus commonly occurs for 24 to 72 hours after abdominal surgery.

Symptoms of postoperative ileus include, e.g., moderate and diffuse abdominal discomfort, constipation, abdominal distension, nausea or vomiting, lack of bowel movement and/or flatulence, and excessive belching.

Treatments for postoperative ileus include, e.g., nil per os (NPO or "Nothing by Mouth") until peristaltic sound is heard from auscultation of the area where this portion lies, nasogastric suction, parenteral feeds, and medications (e.g., lactulose and erythromycin).

The disclosure provides methods of treating (e.g., ameliorating, reducing, or eliminating one or more symptoms of) postoperative ileus by administering a plasma kallikrein binding protein (e.g., a therapeutically effective amount of a plasma kallikrein binding protein) to a subject having or suspected of having postoperative ileus. Additionally provided are methods of treating postoperative ileus by administering a plasma kallikrein binding protein (e.g., a therapeutically effective amount of a plasma kallikrein binding protein) in combination with a second therapy, e.g., nil per os, nasogastric suction, parenteral feeds, and/or a medication (e.g., lactulose and/or erythromycin). The disclosure also provides methods of preventing postoperative ileus or a symptom thereof by administering a plasma kallikrein binding protein (e.g., a prophylactically effective amount of a plasma kallikrein binding protein) to a subject at risk of developing postoperative ileus (e.g., a subject that has had abdominal surgery).

Guidance for the determination of the dosage that delivers a therapeutically effective amount of a plasma kallikrein binding protein may be obtained from animal models of postoperative ileus, such as a model to investigate postoperative ileus with strain gauge transducers in awake rats (Huge et al. J Surg Res. 74(2):112-8 (1998)).

Aortic Aneurysm

An aortic aneurysm is a general term for any swelling (dilatation or aneurysm) of the aorta, usually representing an underlying weakness in the wall of the aorta at that location. Types of aortic aneurysms include aortic root aneurysm, thoracic aortic aneurysm, abdominal aortic aneurysm, and thoracoabdominal aortic aneurysm.

Most intact aortic aneurysms do not produce symptoms. As they enlarge, symptoms of aortic aneurysm include, e.g., anxiety or feeling of stress, nausea or vomiting, clammy skin, rapid heart rate, abdominal pain, back pain may develop, leg pain or numbness, erythema nodosum (leg lesions typically found near the ankle region), and a hoarse voice as the left recurrent laryngeal nerve winding around the arch of the aorta is stretched. Once an aneurysm is ruptured, it can cause severe pain and massive internal hemorrhage, and is fatal in the absence of prompt treatment.

Treatments for aortic aneurysm include, e.g., medications, surgical treatment and endovascular treatment. Smaller aneurysms that are not at high risk for rupturing can be treated with drugs to treat high blood pressure, such as beta-blockers; or doxycycline for matrix metalloproteinase-9 inhibition. Surgical treatment typically involves opening up of the dilated portion of the aorta and insertion of a synthetic (Dacron or Gore-tex) patch tube. Endovascular treatment, as a minimally invasive alternative to open surgery repair, involves the placement of an endovascular stent via a percutaneous technique (usually through the femoral arteries) into the diseased portion of the aorta.

The disclosure provides methods of treating (e.g., stabilizing, reducing, or eliminating one or more symptoms of) aortic aneurysm by administering a plasma kallikrein binding protein (e.g., a therapeutically effective amount of a plasma kallikrein binding protein) to a subject having or suspected of having aortic aneurysm. Additionally provided are methods of treating aortic aneurysm by administering a plasma kallikrein binding protein (e.g., a therapeutically effective amount of a plasma kallikrein binding protein) in combination with a second therapy, e.g., a medication (e.g., a drug to treat high blood pressure (e.g., a beta-blocker) or doxycycline), surgery, and/or an endovascular treatment. The disclosure also provides methods of preventing aortic aneurysm or a symptom thereof by administering a plasma kallikrein binding protein (e.g., a prophylactically effective amount of a plasma kallikrein binding protein) to a subject at risk of developing aortic aneurysm (e.g., a subject that has high blood pressure).

Guidance for the determination of the dosage that delivers a therapeutically effective amount of a plasma kallikrein binding protein may be obtained from an animal model of aortic aneurysm, e.g., a rat model of abdominal aortic aneurysm using a combination of intraluminal elastase infusion and extraluminal calcium chloride exposure (Tanaka et al. J Vasc Surg. 50(6):1423-32 (2009)).

Osteoarthritis

Osteoarthritis, also known as degenerative arthritis, is characterized by the breakdown and eventual loss of the cartilage of one or more joints. Osteoarthritis occurs when the cartilage that cushions the ends of bones in the joints deteriorates over time. The smooth surface of the cartilage becomes rough, causing irritation. If the cartilage wears down completely, the ends of the bones will be damaged. Osteoarthritis commonly affects the hands, feet, spine, and large weight-bearing joints, such as the hips and knees.

Symptoms of osteoarthritis include, e.g., pain, tenderness, stiffness, loss of flexibility, grating sensation, and bone spurs.

Treatments for osteoarthritis include, e.g., conservative measures (e.g., rest, weight reduction, physical and occupational therapy) and medications (e.g., acetaminophen, pain-relieving creams applied to the skin over the joints (e.g., capsaicin, salycin, methyl salicylate, and menthol), non-steroidal anti-inflammatory drugs (NSAIDs) (e.g., aspirin, ibuprofen, nabumetone and naproxen), and Cox-2 inhibitors.

The disclosure provides methods of treating (e.g., stabilizing, reducing, or eliminating one or more symptoms or stabilizing the subject's score on an osteoarthritis scale) osteoarthritis by administering a plasma kallikrein binding protein (e.g., a therapeutically effective amount of a plasma kallikrein binding protein) to a subject having or suspected of having osteoarthritis. Additionally provided are methods of treating osteoarthritis by administering a plasma kallikrein binding protein (e.g., a therapeutically effective amount of a plasma kallikrein binding protein) in combination with a second therapy, e.g., a conservative measure (e.g., rest, weight reduction, physical and/or occupational therapy) and/or a medication (e.g., acetaminophen, a topical pain-relieving cream, an NSAID (e.g., aspirin, ibuprofen, nabumetone, or naproxen), and/or a Cox-2 inhibitor. The disclosure also provides methods of preventing osteoarthritis or a symptom thereof by administering a plasma kallikrein binding protein (e.g., a prophylactically effective amount of a plasma kallikrein binding protein) to a subject at risk of developing osteoarthritis (e.g., a subject that has had a joint injury).

Scales useful for the assessment of osteoarthritis include, e.g., the Knee Injury and Osteoarthritis Outcome Score (KOOS; Roos et al. (1998) J. Orthop. Sports Phys. Ther.

28(2):88-96), Western Ontario and McMaster Universities Osteoarthrtis Index (WOMAC; Roos et al. (2003) *Health Qual. Life Outcomes* 1(1):17), and the 36-item Short Form General Health Scale (SF-36 GHS), as well as other assessment tools known in the art.

Guidance for the determination of the dosage that delivers a therapeutically effective amount of a plasma kallikrein binding protein may be obtained from an animal model of osteoarthritis, e.g., injection of mono-iodoacetate (MIA) into the femorotibial joint of rodents which promotes loss of articular cartilage similar to that noted in human osteoarthritis (Guzman et al. Toxicol Pathol. 31(6):619-24 (2003)), and transection of the anterior cruciate ligament (ACL) in canines to induce osteoarthritis (Fife and Brandt J Clin Invest. 84(5): 1432-1439 (1989)).

Vasculitis

Vasculitis refers to a heterogeneous group of disorders that are characterized by inflammatory destruction of blood vessels. Both arteries and veins can be affected. Lymphangitis is sometimes considered a type of vasculitis. Vasculitis is primarily due to leukocyte migration and resultant damage. Vasculitis can be classified by the underlying cause, the location of the affected vessels, or the type or size of the blood vessels. Vasculitis is associated with a number of additional disorders and conditions, e.g., Kawasaki disease, Behçet's disease, Polyarteritis nodosa, Wegener's granulomatosis, Cryoglobulinemia, Takayasu's arteritis, Churg-Strauss syndrome, Giant cell arteritis (temporal arteritis), Henoch-Schönlein purpura, Rheumatic diseases (e.g., rheumatoid arthritis and systemic lupus erythematosus), cancer (e.g., lymphomas), infections (e.g., hepatitis C), exposure to chemicals and drugs (e.g., amphetamines, cocaine, and anthrax vaccines which contain the Anthrax Protective Antigen as the primary ingredient).

Symptoms of vasculitis include, e.g., fever, weight loss, palpable purpura, livedo reticularis, myalgia or myositis, arthralgia or arthritis, mononeuritis multiplex, headache, stroke, tinnitus, reduced visual acuity, acute visual loss, myocardial infarction, hypertension, gangrene, nose bleeds, bloody cough, lung infiltrates, abdominal pain, bloody stool, perforations, and glomerulonephritis.

Treatments for vasculitis include, e.g., cortisone-related medications (e.g., prednisone) and immune suppression drugs (e.g., cyclophosphamide).

The disclosure provides methods of treating (e.g., stabilizing, reducing, or eliminating one or more symptoms or stabilizing the subject's score on a vasculitis scale) vasculitis by administering a plasma kallikrein binding protein (e.g., a therapeutically effective amount of a plasma kallikrein binding protein) to a subject having or suspected of having vasculitis. Additionally provided are methods of treating vasculitis by administering a plasma kallikrein binding protein (e.g., a therapeutically effective amount of a plasma kallikrein binding protein) in combination with a second therapy (e.g., a cortisone-related medication (e.g., prednisone) and/or an immune suppression drug (e.g., cyclophosphamide)). The disclosure also provides methods of preventing vasculitis or a symptom thereof by administering a plasma kallikrein binding protein (e.g., a prophylactically effective amount of a plasma kallikrein binding protein) to a subject at risk of developing vasculitis (e.g., a subject that has had Kawasaki disease, Behçet's disease, Polyarteritis nodosa, Wegener's granulomatosis, Cryoglobulinemia, or Takayasu's arteritis, and so forth).

The disclosure also provides methods of treating (e.g., stabilizing, reducing, or eliminating one or more symptoms or stabilizing the subject's score on a vasculitis scale) vasculitis associated with systemic lupus erythematosis by administering a plasma kallikrein binding protein (e.g., a therapeutically effective amount of a plasma kallikrein binding protein) to a subject having or suspected of having vasculitis associated with systemic lupus erythematosis. Additionally provided are methods of treating vasculitis associated with systemic lupus erythematosis by administering a plasma kallikrein binding protein (e.g., a therapeutically effective amount of a plasma kallikrein binding protein) in combination with a second therapy, e.g., a cortisone-related medication (e.g., prednisone) and/or an immune suppression drug (e.g., cyclophosphamide).

Further provided are methods of treating (e.g., ameliorating, stabilizing, or eliminating one or more symptoms) a vasculitis associated disorder (Kawasaki disease, Behçet's disease, Polyarteritis nodosa, Wegener's granulomatosis, Cryoglobulinemia, Takayasu's arteritis, Churg-Strauss syndrome, Giant cell arteritis (temporal arteritis), Henoch-Schonlein purpura, Rheumatic diseases (e.g., rheumatoid arthritis and systemic lupus erythematosus), cancer (e.g., lymphomas), infections (e.g., hepatitis C), exposure to chemicals and drugs (e.g., amphetamines, cocaine, and anthrax vaccines which contain the Anthrax Protective Antigen as the primary ingredient)) by administering a plasma kallikrein binding protein (e.g., a therapeutically effective amount of a plasma kallikrein binding protein) to a subject having or suspected of having a vasculitis associated disorder. The disclosure also provides methods of preventing a vasculitis associated disorder or a symptom thereof by administering a plasma kallikrein binding protein (e.g., a prophylactically effective amount of a plasma kallikrein binding protein) to a subject at risk of developing a vasculitis associated disorder.

Scales useful for the assessment of osteoarthritis include, e.g., Birmingham Vasculitis Activity score (BVAS) version 3 (Mukhtyar et al. Ann Rheum Dis. 68(12):1827-32 (2009)), as well as other assessment tools known in the art.

Guidance for the determination of the dosage that delivers a therapeutically effective amount of a plasma kallikrein binding protein may be obtained from an animal model of vasculitis, see e.g., those described in Katz et al., Clin Rev Allergy Immunol. 35(1-2):11-8 (2008) and references cited therein.

Head Trauma

Head trauma refers to trauma to the head, which may or may not include injury to the brain. Types of head trauma include concussion, epidural hematoma, subdural hematoma, cerebral contusion, and diffuse axonal injury.

Symptoms of head trauma include, e.g., coma, confusion, drowsiness, personality change, seizures, nausea and vomiting, headache and a lucid interval, during which a patient appears conscious only to deteriorate later, leaking cerebrospinal fluid, visible deformity or depression in the head or face, an eye that cannot move or is deviated to one side can indicate that a broken facial bone is pinching a nerve that innervates eye muscles, wounds or bruises on the scalp or face, basilar skull fractures, a subcutaneous bleed over the mastoid, hemotympanum, cerebrospinal fluid rhinorrhea, and otorrhea.

Treatments for head trauma include, e.g., controlling elevated intracranial pressure (e.g., sedation, paralytics, cerebrospinal fluid diversion), decompressive craniectomy, barbiturate coma, hypertonic saline, and hypothermia.

The disclosure provides methods of treating (e.g., stabilizing, reducing, or eliminating one or more symptoms or stabilizing the subject's score on a head trauma scale) head trauma by administering a plasma kallikrein binding protein (e.g., a therapeutically effective amount of a plasma kallikrein binding protein) to a subject having or suspected of having head trauma. Additionally provided are methods of treating head trauma by administering a plasma kallikrein binding protein (e.g., a therapeutically effective amount of a plasma kallikrein binding protein) in combination with a second therapy, e.g., controlling elevated intracranial pressure (e.g., sedation, a paralytic, and/or cerebrospinal fluid diversion), decompressive craniectomy, barbiturate coma, hypertonic saline, and/or hypothermia. The disclosure also provides methods of preventing head trauma or a symptom thereof by administering a plasma kallikrein binding protein (e.g., a prophylactically effective amount of a plasma kallikrein binding protein) to a subject at risk of developing head trauma (e.g., a subject that will be participating in a dangerous activity or contact sport).

Scales useful for assessing head trauma and symptoms of head trauma include, e.g., the Glasgow Coma Scale (Teasdale and Jennett, Lancet 13; 2(7872):81-4 (1974)), as well as other assessment tools known in the art.

Guidance for the determination of the dosage that delivers a therapeutically effective amount of a plasma kallikrein binding protein may be obtained from animal models of head trauma, see, e.g., those described in Cernak, NeuroRx. 2(3): 410-422 (2005) and references cited therein.

Brain Edema

Brain edema (cerebral edema) is an excess accumulation of water in the intracellular and/or extracellular spaces of the brain. Types of brain edema include, e.g., vasogenic cerebral edema, cytotoxic cerebral edema, osmotic cerebral edema, and interstitial cerebral edema.

Vasogenic cerebral edema is due to a breakdown of tight endothelial junctions which make up the blood-brain barrier (BBB). This allows normally excluded intravascular proteins and fluid to penetrate into cerebral parenchymal extracellular space. Once plasma constituents cross the BBB, the edema spreads; this may be quite fast and widespread. As water enters white matter it moves extracellularly along fiber tracts and can also affect the gray matter. This type of edema is seen in response to trauma, tumors, focal inflammation, late stages of cerebral ischemia and hypertensive encephalopathy. Some of the mechanisms contributing to BBB dysfunction are: physical disruption by arterial hypertension or trauma, tumor-facilitated release of vasoactive and endothelial destructive compounds (e.g., arachidonic acid, excitatory neurotransmitters, eicosanoids, bradykinin, histamine and free radicals). Some of the special subcategories of vasogenic edema include: hydrostatic cerebral edema, cerebral edema from brain cancer, high altitude cerebral edema.

Cytotoxic cerebral edema is due to the derangement in cellular metabolism resulting in inadequate functioning of the sodium and potassium pump in the glial cell membrane. As a result there is cellular retention of sodium and water. Cytotoxic edema is seen with various intoxications (dinitrophenol, triethyltin, hexachlorophene, isoniazid), in Reye's syndrome, severe hypothermia, early ischemia, encephalopathy, early stroke or hypoxia, cardiac arrest, pseudotumor cerebri, and cerebral toxins.

Osmotic cerebral edema occurs when plasma is diluted by excessive water intake (or hyponatremia), syndrome of inappropriate antidiuretic hormone secretion (SIADH), hemodialysis, or rapid reduction of blood glucose in hyperosmolar hyperglycemic state (HHS), formerly hyperosmolar non-ketotic acidosis (HONK) and brain osmolality exceeds the serum osmolality creating an abnormal pressure.

Interstitial cerebral edema occurs in obstructive hydrocephalus. This form of edema is due to rupture of cerebral-spinal fluid (CSF)-brain barrier resulting in trans-ependymal flow of CSF, which permits CSF to penetrate brain and spread in the extracellular space of white matter.

Symptoms of brain edema (e.g., peritumoral brain edema) include, e.g., headache, loss of coordination (ataxia), weakness, and decreasing levels of consciousness including disorientation, loss of memory, hallucinations, psychotic behavior, and coma.

Treatments for brain edema (e.g., peritumoral brain edema) include, e.g., medications (e.g. dexamethasone, mannitol, diuretics) and surgical decompression.

The disclosure provides methods of treating (e.g., stabilizing, reducing, or eliminating one or more symptoms of) brain edema (e.g., peritumoral brain edema) by administering a plasma kallikrein binding protein (e.g., a therapeutically effective amount of a plasma kallikrein binding protein) to a subject having or suspected of having brain edema (e.g., peritumoral brain edema). Additionally provided are methods of treating brain edema (e.g., peritumoral brain edema) by administering a plasma kallikrein binding protein (e.g., a therapeutically effective amount of a plasma kallikrein binding protein) in combination with a second therapy, e.g., a medication (e.g. dexamethasone, mannitol, and/or diuretics) and/or surgical decompression. The disclosure also provides methods of preventing brain edema or a symptom thereof by administering a plasma kallikrein binding protein (e.g., a prophylactically effective amount of a plasma kallikrein binding protein) to a subject at risk of developing brain edema (e.g., a subject that has been diagnosed with a brain tumor).

Guidance for the determination of the dosage that delivers a therapeutically effective amount of a plasma kallikrein binding protein may be obtained from animal models of brain edema, e.g., a rat model of cerebral embolism in which recirculation can be introduced in the ischemic area (Koizumi et al., Jpn J Stroke 8: 1-8 (1986)).

Sepsis

Sepsis is a serious medical condition that is characterized by a whole-body inflammatory state and the presence of a known or suspected infection. This immunological response may be caused by microbes in the blood, urine, lungs, skin, or other tissues and can lead to widespread activation of acute-phase proteins, affecting the complement system and the coagulation pathways, which then cause damage to the vasculature as well as to the organs. Different levels of sepsis include systemic inflammatory response syndrome (SIRS), sepsis (SIRS in response to a confirmed infectious process), severe sepsis (sepsis with organ dysfunction, hypoperfusion, or hypotension), and septic shock (sepsis with refractory arterial hypotension or hypoperfusion abnormalities in spite of adequate fluid resuscitation).

Symptoms of sepsis include, e.g., general symptoms related to the infection, acute inflammation present throughout the entire body, hypothermia or fever, tachycardia, tachypnea or hypocapnia due to hyperventilation, leukopenia, leukocytosis, bandemia, and organ (e.g., lung, brain, liver, kidney, and/or heart) dysfunction.

Treatments for sepsis include, e.g., antibiotics, vasopressor drugs, insulin, corticosteroids, drotrecogin alfa, surgical drainage of infected fluid collections, fluid replacement, and appropriate support for organ dysfunction (e.g., hemodialysis in kidney failure, mechanical ventilation in pulmonary dysfunction, transfusion of blood products, and drug and fluid therapy for circulatory failure). Early Goal Directed Therapy (EGDT), a systematic approach to resuscitation, can be used to treat severe sepsis and septic shock.

The disclosure provides methods of treating (e.g., stabilizing, reducing, or eliminating one or more symptoms or stabilizing the subject's score on a sepsis scale) sepsis by administering a plasma kallikrein binding protein (e.g., a therapeutically effective amount of a plasma kallikrein binding protein) to a subject having or suspected of having sepsis. Additionally provided are methods of treating sepsis by administering a plasma kallikrein binding protein (e.g., a therapeutically effective amount of a plasma kallikrein binding protein) in combination with a second therapy, e.g., an antibiotic, a vasopressor drug, insulin, a corticosteroid, drotrecogin alfa, surgical drainage of infected fluid collections, fluid replacement, an appropriate support for organ dysfunction (e.g., hemodialysis in kidney failure, mechanical ventilation in pulmonary dysfunction, transfusion of blood products, and/or drug and fluid therapy for circulatory failure), and/or an Early Goal Directed Therapy (EGDT). The disclosure also provides methods of preventing sepsis or a symptom thereof by administering a plasma kallikrein binding protein (e.g., a prophylactically effective amount of a plasma kallikrein binding protein) to a subject at risk of developing sepsis (e.g., a subject that has been diagnosed as having an infection).

Scales useful for assessing sepsis and symptoms of sepsis include, e.g., the Baltimore Sepsis Scale (Meek et al. *J Burn Care Rehabil.* 12(6):564-8 (1991)) as well as other assessment tools known in the art.

Guidance for the determination of the dosage that delivers a therapeutically effective amount of a plasma kallikrein binding protein may be obtained from animal models of sepsis, see, e.g., those described in U.S. Pat. No. 6,964,856, and Buras et al. Nat Rev Drug Discov. 4(10):854-65 (2005) and references cited therein.

Acute Middle Cerebral Artery (MCA) Ischemic Event (Stroke)

An acute middle cerebral artery (MCA) ischemic event (stroke) is the rapidly developing loss of brain function(s) due to disturbance in the blood supply to the brain due to ischemia (lack of glucose and oxygen supply) caused by thrombosis (e.g., venous thrombosis), embolism, or systemic hypoperfusion. As a result, the affected area of the brain is unable to function, leading to inability to move one or more limbs on one side of the body, inability to understand or formulate speech, or inability to see one side of the visual field. A stroke is a medical emergency and can cause permanent neurological damage, complications, and/or death.

Symptoms of acute middle cerebral artery (MCA) ischemic event (stroke) include, e.g., hemiplegia, decreased sensation and muscle weakness of the face, numbness, reduction in sensory or vibratory sensation, altered smell, taste, hearing or vision (total or partial), drooping of eyelid (ptosis) and weakness of ocular muscles, decreased reflexes, balance problems and nystagmus, altered breathing and heart rate, weakness in sternocleidomastoid muscle with inability to turn head to one side, weakness in tongue (inability to protrude and/or move from side to side), aphasia, apraxia, visual field defect, memory deficits, hemineglect, disorganized thinking, confusion, hypersexual gestures, anosognosia, trouble walking, altered movement coordination, and vertigo and/or disequilibrium.

Treatment for acute middle cerebral artery (MCA) ischemic event (stroke) includes, e.g., thrombolysis (e.g., tissue plasminogen activator (tPA)), thrombectomy, angioplasty and stenting, therapeutic hypothermia, and medications (e.g., aspirin, clopidogrel and dipyridamole).

The disclosure provides methods of treating (e.g., stabilizing, reducing, or eliminating one or more symptoms or stabilizing the subject's score on a stroke scale) acute middle cerebral artery (MCA) ischemic event (stroke) by administering a plasma kallikrein binding protein (e.g., a therapeutically effective amount of a plasma kallikrein binding protein) to a subject having or suspected of having acute middle cerebral artery (MCA) ischemic event (stroke). Additionally provided are methods of treating acute middle cerebral artery (MCA) ischemic event (stroke) by administering a plasma kallikrein binding protein (e.g., a therapeutically effective amount of a plasma kallikrein binding protein) in combination with a second therapy, e.g., thrombolysis (e.g., tissue plasminogen activator (tPA)), thrombectomy, angioplasty and stenting, therapeutic hypothermia, and/or a medication (e.g., aspirin, clopidogrel and dipyridamole). The disclosure also provides methods of preventing acute middle cerebral artery (MCA) ischemic event (stroke) or a symptom thereof by administering a plasma kallikrein binding protein (e.g., a prophylactically effective amount of a plasma kallikrein binding protein) to a subject at risk of developing acute middle cerebral artery (MCA) ischemic event (stroke) (e.g., a subject that has experienced systemic hypoperfusion).

Scales useful for assessing acute middle cerebral artery (MCA) ischemic event (stroke) and symptoms of acute middle cerebral artery (MCA) ischemic event (stroke) include, e.g., Oxford Community Stroke Project classification (OCSP, also known as the Bamford or Oxford classification) (Bamford et al., Lancet 337 (8756): 1521-6 (1991)), and TOAST (Trial of Org 10172 in Acute Stroke Treatment) (Adams et al., *Stroke* 24 (1): 35-41 (1993)).

Guidance for the determination of the dosage that delivers a therapeutically effective amount of a plasma kallikrein binding protein may be obtained from animal models of acute middle cerebral artery (MCA) ischemic event (stroke), see, e.g., those described in Beech et al., Brain Res 895: 18-24 (2001), Buchan et al., Stroke 23 (2): 273-9 (1992), Carmichael, NeuroRx 2: 396-409 (2005), Chen et al., Stroke 17 (4): 738-43 (1986), Dittmar et al., Stroke 34: 2252-7 (2003), Dittmar et al., J Neurosci Methods 156: 50 (2006), Gerriets et al., J Neurosci Methods 122: 201-11 (2003), Gerriets et al., Stroke 35: 2372-2377 (2004), Graham et al., Comp Med 54: 486-496 (2004), Koizumi et al., Jpn J Stroke 8: 1-8 (2004), Longa et al., Stroke 20 (1): 84-91 (1989), Mayzel-Oreg, Magn Reson Med 51: 1232-8 (2004), Schmid-Elsaesser et al., Stroke 29 (10): 2162-70 (1989), Tamura et al., J Cereb Blood Flow Metab 1: 53-60 (1981), Watson et al., Ann Neurol 17: 497-504 (1985), and Zhang et al., J Cereb Blood Flow Metab 17: 123-35 (1997).

Restenosis

Restenosis is the reoccurrence of stenosis, a narrowing of a blood vessel, leading to restricted blood flow. Restenosis usually pertains to an artery or other large blood vessel that has become narrowed, received treatment to clear the blockage such as angioplasty, and subsequently become renarrowed. It can be defined as a reduction in the circumference of the lumen of 50% or more, and had a high incidence rate (25-50%) in patients who had undergone balloon angioplasty, with the majority of patients needing further angioplasty within 6 months.

Treatments for restenosis include, e.g., additional angioplasty if restenosis occurs without a stent or at either end of a stent, repeated angioplasty and insertion of another stent inside the original if restenosis occurs within a stent, drug-eluted stents, brachytherapy, and intracoronary radiation.

The disclosure provides methods of treating (e.g., stabilizing, reducing, or eliminating one or more symptoms of) restenosis (e.g., after angioplasty) by administering a plasma kallikrein binding protein (e.g., a therapeutically effective amount of a plasma kallikrein binding protein) to a subject having or suspected of having restenosis (e.g., after angioplasty). Additionally provided are methods of treating restenosis (e.g., after angioplasty) by administering a plasma kallikrein binding protein (e.g., a therapeutically effective amount of a plasma kallikrein binding protein) in combination with a second therapy, e.g., angioplasty if restenosis occurs without a stent or at either end of a stent, repeated angioplasty and insertion of another stent inside the original if restenosis occurs within a stent, a drug-eluted stent, brachytherapy, and/or intracoronary radiation. The disclosure also provides methods of preventing restenosis or a symptom thereof by administering a plasma kallikrein binding protein (e.g., a prophylactically effective amount of a plasma kallikrein binding protein) to a subject at risk of developing restenosis (e.g., a subject that has had stenosis).

Guidance for the determination of the dosage that delivers a therapeutically effective amount of a plasma kallikrein binding protein may be obtained from animal models of restenosis, see, e.g., those described in U.S. Pat. Nos. 5,304,122 and 6,034,053, and Kantor et al., Cardiovasc Radiat Med. 1(1):48-54 (1999), and references cited therein.

Systemic Lupus Erythematosus Nephritis

Systemic lupus erythematosus nephritis is an inflammation of the kidney caused by systemic lupus erythematosus (SLE), a chronic autoimmune connective tissue disease. SLE can be associated with vasculitis which are disorders characterized by inflammatory destruction of blood vessels.

Symptoms of systemic lupus erythematosus nephritis include, e.g., general symptoms of kidney disease, weight gain, high blood pressure, darker foamy urine, and swelling around the eyes, legs, ankles or fingers.

Treatments for systemic lupus erythematosus nephritis include, e.g., steroid therapy (e.g., corticosteroids), chemotherapy (e.g., cyclophosphamide, azathioprine, mycophenolate mofetil, or cyclosporine), and immunosuppressant agents (e.g., mycophenolate mofetil and intravenous cyclophosphamide).

The disclosure provides methods of treating (e.g., stabilizing, reducing, or eliminating one or more symptoms or stabilizing the subject's score on a lupus scale) systemic lupus erythematosus nephritis by administering a plasma kallikrein binding protein (e.g., a therapeutically effective amount of a plasma kallikrein binding protein) to a subject having or suspected of having systemic lupus erythematosus nephritis. Additionally provided are methods of treating systemic lupus erythematosus nephritis by administering a plasma kallikrein binding protein (e.g., a therapeutically effective amount of a plasma kallikrein binding protein) in combination with a second therapy, e.g., steroid therapy (e.g., a corticosteroid), chemotherapy (e.g., cyclophosphamide, azathioprine, mycophenolate mofetil, and/or cyclosporine), and/or an immunosuppressant agent (e.g., mycophenolate mofetil and/or intravenous cyclophosphamide). The disclosure also provides methods of preventing systemic lupus erythematosus nephritis or a symptom thereof by administering a plasma kallikrein binding protein (e.g., a prophylactically effective amount of a plasma kallikrein binding protein) to a subject at risk of developing systemic lupus erythematosus nephritis (e.g., a subject that has been diagnosed with lupus or a subject having a family member with lupus or a genetic predisposition thereto).

Scales useful for assessing systemic lupus erythematosus nephritis and symptoms of systemic lupus erythematosus nephritis include, e.g., World Health Organization (WHO) classification based on the biopsy (Weening et al., *J. Am. Soc. Nephrol.* 15 (2): 241-50 (2004)) as well as other assessment tools known in the art.

Guidance for the determination of the dosage that delivers a therapeutically effective amount of a plasma kallikrein binding protein may be obtained from animal models of systemic lupus erythematosus nephritis, see, e.g., those described in U.S. Pat. No. 7,265,261, Peng, Methods Mol. Med. 102:227-72 (2004), and references cited therein.

Burn Injury and Wound Healing

A burn injury is a type of injury that may be caused by heat, electricity, chemicals, light, radiation, or friction. Muscle, bone, blood vessel, dermal and epidermal tissue can all be damaged with subsequent pain due to profound injury to nerves. Depending on the location affected and the degree of severity, a burn victim may experience a wide number of potentially fatal complications including shock, infection, electrolyte imbalance and respiratory distress. In burn injuries, the damage to epidermis and dermal elements is the result of several key insults which can be divided into initial (e.g., heat injury, inflammatory mediator injury, ischemia induced injury) and delayed insults. Excess heat causes rapid protein denaturation and cell damage. Much of the tissue damage, e.g., in the perfused subsurface burn, can be caused by toxic mediators of inflammation (e.g., oxidants and/or proteases) which are activated with the burn. Consumption of wound oxygen by neutrophils can lead to tissue hypoxia. Instant surface vascular thrombosis occurs along with cell death from the heat insult and causes ischemia and further tissue damage. Delayed injury after the initial heat and mediator damage includes, e.g., inflammation caused by neurotic tissue, bacteria on surface, caustic topical agents, and surface exudate; and continued damage to viable cells and new tissue growth by excess wound proteolytic activity and oxidant release.

Treatments of burn injury include, e.g., intravenous fluids, dressings, pain management (e.g., analgesics (e.g., ibuprofen and acetaminophen), narcotics, and local anesthetics), inflammatory mediator inhibitors, and antibiotics.

The disclosure provides methods of treating (e.g., stabilizing, reducing, or eliminating one or more symptoms or stabilizing the subject's score on a burn scale) a burn injury and/or promoting wound healing by administering a plasma kallikrein binding protein (e.g., a therapeutically effective amount of a plasma kallikrein binding protein) to a subject having or suspected of having a burn injury. Additionally provided are methods of treating a burn injury by administering a plasma kallikrein binding protein (e.g., a therapeutically effective amount of a plasma kallikrein binding protein) in combination with a second therapy, e.g., intravenous fluid, a dressing, pain management (e.g., an analgesic (e.g., ibuprofen and acetaminophen), a narcotic, and a local anesthetic), an inflammatory mediator inhibitor, and an antibiotic. The disclosure also provides methods of preventing burn injuries or a symptom thereof by administering a plasma kallikrein binding protein (e.g., a prophylactically effective amount of a plasma kallikrein binding protein) to a subject at risk of developing burn injuries (e.g., a subject whose occupation creates a risk of a burn injury, e.g., firefighter or cook).

Scales useful for assessing burns and symptoms of burns include, e.g., burn scales by degrees, by thickness, and by total body surface area (TBSA) (Meek et al. *J Burn Care Rehabil.* 12(6):564-8 (1991)) as well as other assessment tools known in the art.

Guidance for the determination of the dosage that delivers a therapeutically effective amount of a plasma kallikrein binding protein may be obtained from animal models of burn, such as a porcine burn model (Singer and McClain, Methods Mol. Med. 78:107-19 (2003), a sheep model of thermal injury (Jonkam et al., Shock, 28:704-709 (2007)), a rabbit model of thermal injury (Nwariaku et al., Burns, 22:324-327 (1996)), and a mouse model of burn wounding (Stevenson et al., Methods Mol Med. 78:95-105 (2003)).

Combination Therapies

A plasma kallikrein binding protein described herein, e.g., an anti-plasma kallikrein antibody, e.g., an anti-plasma kallikrein Fab or IgG, can be administered in combination with one or more of the other therapies for treating a disease or condition associated with plasma kallikrein activity, e.g., a disease or condition described herein. For example, a plasma kallikrein binding protein can be used therapeutically or prophylactically with surgery, another anti-plasma kallikrein Fab or IgG (e.g., another Fab or IgG described herein), another plasma kallikrein inhibitor, a peptide inhibitor, or small molecule inhibitor. Examples of plasma kallikrein inhibitors that can be used in combination therapy with a plasma kallikrein binding protein described herein include plasma kallikrein inhibitors described in, e.g., WO 95/21601 or WO 2003/103475.

One or more plasma kallikrein inhibitors can be used in combination with one or more plasma kallikrein binding proteins described herein. For example, the combination can result in a lower dose of the inhibitor being needed, such that side effects are reduced.

A plasma kallikrein binding protein described herein can be administered in combination with one or more current therapies for treating a plasma kallikrein associated disease or condition, including, but not limited to the current therapies for treating the disorder, e.g., a current therapy for rheumatoid arthritis, gout, intestinal bowel disease, oral mucositis, neuropathic pain, inflammatory pain, spinal stenosis-degenerative spine disease, arterial or venous thrombosis, post operative ileus, aortic aneurysm, osteoarthritis, vasculitis, head trauma or peri-tumor brain edema, sepsis, acute middle cerebral artery (MCA) ischemic event (stroke), restenosis (e.g., after angioplasty), systemic lupus erythematosis nephritis, burn injury, or wound healing. For example, pKal inhibition is a novel mechanism of treating disease and therefore could provide effects that are synergistic or additive with other therapeutics. For example, a protein described herein that inhibits plasma kallikrein or that inhibits a downstream event of plasma kallikrein activity can also be used in combination with another treatment for a plasma kallikrein associated disease, such as surgery or administration of a second agent, e.g., as described herein. For example, the second agent can include ecallantide, a C1 esterase inhibitor (e.g., CINRYZE™), aprotinin (TRASYLOL®), a bradykinin B2 receptor inhibitor (e.g., icatibant (FIRAZYR®)).

The term "combination" refers to the use of the two or more agents or therapies to treat the same patient, wherein the use or action of the agents or therapies overlap in time. The agents or therapies can be administered at the same time (e.g., as a single formulation that is administered to a patient or as two separate formulations administered concurrently) or sequentially in any order. Sequential administrations are administrations that are given at different times. The time between administration of the one agent and another agent can be minutes, hours, days, or weeks. The use of a plasma kallikrein binding protein described herein can also be used to reduce the dosage of another therapy, e.g., to reduce the side effects associated with another agent that is being administered. Accordingly, a combination can include administering a second agent at a dosage at least 10, 20, 30, or 50% lower than would be used in the absence of the plasma kallikrein binding protein.

The second agent or therapy can also be another agent for a plasma kallikrein associated therapy. Non-limiting examples of another treatment for a plasma kallikrein associated disease or condition include, e.g., ecallantide, a C1 esterase inhibitor (e.g., CINRYZE™), aprotinin (TRASYLOL®), a bradykinin B2 receptor inhibitor (e.g., icatibant (FIRAZYR®)) or a second binding protein described herein.

A combination therapy can include administering an agent that reduces the side effects of other therapies. The agent can be an agent that reduces the side effects of a plasma kallikrein associated disease treatment. For example, for inflammatory diseases, a pKal inhibitor could be steroid sparring. Also, there could be synergism with a TNF-alpha inhibitor for treating inflammation or a VEGF blocker for treating cancer and/or angiogenesis.

Diagnostic Uses

A protein that binds to plasma kallikrein described herein can have in vitro and in vivo diagnostic utilities. A plasma kallikrein binding protein described herein (e.g., a protein that binds or binds and inhibits plasma kallikrein) can be used, e.g., for in vivo imaging, e.g., during a course of treatment for a disease or condition in which plasma kallikrein is active, e.g., a disease or condition described herein, or in diagnosing a disease or condition described herein.

In one aspect, the disclosure provides a diagnostic method for detecting the presence of plasma kallikrein, in vitro or in vivo (e.g., in vivo imaging in a subject). The method can include localizing plasma kallikrein within a subject or within a sample from a subject. With respect to sample evaluation, the method can include, for example: (i) contacting a sample with plasma kallikrein binding protein; and (ii) detecting the location of the plasma kallikrein binding protein in the sample.

A plasma kallikrein binding protein can also be used to determine the qualitative or quantitative level of expression of plasma kallikrein in a sample. The method can also include contacting a reference sample (e.g., a control sample, e.g., a negative control) with the binding protein, and determining a corresponding assessment of the reference sample. A difference (e.g., increase), e.g., a statistically significant difference, in the formation of the complex in the sample or subject relative to the control sample or subject can be indicative of the presence of plasma kallikrein in the sample. In one embodiment, the plasma kallikrein binding protein does not cross react with another kallikrein protein, such as tissue kallikrein and/or with plasma prekallikrein. E.g., the binding protein binds to another kallikrein protein or to prekallikrein 5- to 10-fold less well (or even less well) than it binds to plasma kallikrein. For example, the binding protein can bind to plasma kallikrein with a KD of ~10-50 pM, whereas it binds to tissue kallikrein and/or prekallikrein at ~10 nM.

The plasma kallikrein binding protein can be directly or indirectly labeled with a detectable substance to facilitate detection of the bound or unbound antibody. Suitable detectable substances include various enzymes, prosthetic groups, fluorescent materials, luminescent materials and radioactive materials.

Complex formation between the plasma kallikrein binding protein and plasma kallikrein can be detected by evaluating the binding protein bound to the plasma kallikrein or unbound binding protein. Conventional detection assays can be used, e.g., an enzyme-linked immunosorbent assays (ELISA), a radioimmunoassay (RIA) or tissue immunohistochemistry. Further to labeling the plasma kallikrein binding protein, the presence of plasma kallikrein can be assayed in a sample by a competition immunoassay utilizing standards labeled with a detectable substance and an unlabeled plasma kallikrein binding protein. In one example of this assay, the biological sample, the labeled standards, and the plasma kallikrein binding protein are combined and the amount of labeled standard bound to the unlabeled binding protein is determined. The amount of plasma kallikrein in the sample is inversely proportional to the amount of labeled standard bound to the plasma kallikrein binding protein.

Fluorophore and chromophore labeled proteins can be prepared. Because antibodies and other proteins absorb light having wavelengths up to about 310 nm, the fluorescent moieties should be selected to have substantial absorption at wavelengths above 310 nm and preferably above 400 nm. A variety of suitable fluorescers and chromophores are described by Stryer, 1968, Science 162:526 and Brand, L. et al., 1972, Annu. Rev. Biochem. 41:843-868. The proteins can be labeled with fluorescent chromophore groups by conventional procedures such as those disclosed in U.S. Pat. Nos. 3,940,475, 4,289,747, and 4,376,110. One group of fluorescers having a number of the desirable properties described above is the xanthene dyes, which include the fluoresceins and rhodamines Another group of fluorescent compounds are the naphthylamines. Once labeled with a fluorophore or chromophore, the protein can be used to detect the presence or localization of the plasma kallikrein in a sample, e.g., using fluorescent microscopy (such as confocal or deconvolution microscopy).

Histological Analysis.

Immunohistochemistry can be performed using the proteins described herein. For example, in the case of an antibody, the antibody can be synthesized with a label (such as a purification or epitope tag), or can be detectably labeled, e.g., by conjugating a label or label-binding group. For example, a chelator can be attached to the antibody. The antibody is then contacted to a histological preparation, e.g., a fixed section of tissue that is on a microscope slide. After an incubation for binding, the preparation is washed to remove unbound antibody. The preparation is then analyzed, e.g., using microscopy, to identify if the antibody bound to the preparation.

Of course, the antibody (or other polypeptide or peptide) can be unlabeled at the time of binding. After binding and washing, the antibody is labeled in order to render it detectable.

Protein Arrays.

The plasma kallikrein binding protein can also be immobilized on a protein array. The protein array can be used as a diagnostic tool, e.g., to screen medical samples (such as isolated cells, blood, sera, biopsies, and the like). Of course, the protein array can also include other binding proteins, e.g., that bind to plasma kallikrein or to other target molecules.

Methods of producing polypeptide arrays are described, e.g., in De Wildt et al., 2000, Nat. Biotechnol. 18:989-994; Lueking et al., 1999, Anal. Biochem. 270:103-111; Ge, 2000, Nucleic Acids Res. 28, e3, I-VII; MacBeath and Schreiber, 2000, Science 289:1760-1763; WO 01/40803 and WO 99/51773A1. Polypeptides for the array can be spotted at high speed, e.g., using commercially available robotic apparati, e.g., from Genetic MicroSystems or BioRobotics. The array substrate can be, for example, nitrocellulose, plastic, glass, e.g., surface-modified glass. The array can also include a porous matrix, e.g., acrylamide, agarose, or another polymer.

For example, the array can be an array of antibodies, e.g., as described in De Wildt, supra. Cells that produce the proteins can be grown on a filter in an arrayed format. Polypeptide production is induced, and the expressed polypeptides are immobilized to the filter at the location of the cell. A protein array can be contacted with a labeled target to determine the extent of binding of the target to each immobilized polypeptide. Information about the extent of binding at each address of the array can be stored as a profile, e.g., in a computer

database. The protein array can be produced in replicates and used to compare binding profiles, e.g., of a target and a non-target.

FACS (Fluorescence Activated Cell Sorting).

The plasma kallikrein binding protein can be used to label cells, e.g., cells in a sample (e.g., a patient sample). The binding protein is also attached (or attachable) to a fluorescent compound. The cells can then be sorted using fluorescence activated cell sorter (e.g., using a sorter available from Becton Dickinson Immunocytometry Systems, San Jose Calif.; see also U.S. Pat. Nos. 5,627,037; 5,030,002; and 5,137,809). As cells pass through the sorter, a laser beam excites the fluorescent compound while a detector counts cells that pass through and determines whether a fluorescent compound is attached to the cell by detecting fluorescence. The amount of label bound to each cell can be quantified and analyzed to characterize the sample.

The sorter can also deflect the cell and separate cells bound by the binding protein from those cells not bound by the binding protein. The separated cells can be cultured and/or characterized.

In Vivo Imaging.

Also featured is a method for detecting the presence of plasma kallikrein expressing tissues in vivo. The method includes (i) administering to a subject (e.g., a patient having, e.g., a plasma kallikrein associated disease or condition) an anti-plasma kallikrein antibody, conjugated to a detectable marker; (ii) exposing the subject to a means for detecting said detectable marker to the plasma kallikrein expressing tissues or cells. For example, the subject is imaged, e.g., by NMR or other tomographic means.

Examples of labels useful for diagnostic imaging include radiolabels such as ^{131}I, ^{111}In, ^{123}I, ^{99m}Tc, ^{32}P, ^{125}I, ^{3}H, ^{14}C, and ^{188}Rh, fluorescent labels such as fluorescein and rhodamine, nuclear magnetic resonance active labels, positron emitting isotopes detectable by a positron emission tomography ("PET") scanner, chemiluminescers such as luciferin, and enzymatic markers such as peroxidase or phosphatase. Short range radiation emitters, such as isotopes detectable by short range detector probes can also be employed. The protein can be labeled with such reagents; for example, see Wensel and Meares, 1983, Radioimmunoimaging and Radioimmunotherapy, Elsevier, New York for techniques relating to the radiolabeling of antibodies and D. Colcher et al., 1986, *Meth. Enzymol.* 121: 802-816.

The binding protein can be labeled with a radioactive isotope (such as ^{14}C, ^{3}H, ^{35}S, ^{125}I, ^{32}P, ^{131}I). A radiolabeled binding protein can be used for diagnostic tests, e.g., an in vitro assay. The specific activity of a isotopically-labeled binding protein depends upon the half life, the isotopic purity of the radioactive label, and how the label is incorporated into the antibody.

In the case of a radiolabeled binding protein, the binding protein is administered to the patient, is localized to cells bearing the antigen with which the binding protein reacts, and is detected or "imaged" in vivo using known techniques such as radionuclear scanning using e.g., a gamma camera or emission tomography. See e.g., A. R. Bradwell et al., "Developments in Antibody Imaging", Monoclonal Antibodies for Cancer Detection and Therapy, R. W. Baldwin et al., (eds.), pp 65 85 (Academic Press 1985). Alternatively, a positron emission transaxial tomography scanner, such as designated Pet VI located at Brookhaven National Laboratory, can be used where the radiolabel emits positrons (e.g., ^{11}C, ^{18}F, ^{15}O, and ^{13}N).

MRI Contrast Agents.

Magnetic Resonance Imaging (MRI) uses NMR to visualize internal features of living subject, and is useful for prognosis, diagnosis, treatment, and surgery. MRI can be used without radioactive tracer compounds for obvious benefit. Some MRI techniques are summarized in EP-A-0 502 814. Generally, the differences related to relaxation time constants T1 and T2 of water protons in different environments are used to generate an image. However, these differences can be insufficient to provide sharp high resolution images.

The differences in these relaxation time constants can be enhanced by contrast agents. Examples of such contrast agents include a number of magnetic agents paramagnetic agents (which primarily alter T1) and ferromagnetic or superparamagnetic (which primarily alter T2 response). Chelates (e.g., EDTA, DTPA and NTA chelates) can be used to attach (and reduce toxicity) of some paramagnetic substances (e.g., Fe^{+3}, Mn^{+2}, Gd^{+3}). Other agents can be in the form of particles, e.g., less than 10 mm to about 10 nM in diameter). Particles can have ferromagnetic, antiferromagnetic, or superparamagnetic properties. Particles can include, e.g., magnetite (Fe_3O_4), γ-Fe_2O_3, ferrites, and other magnetic mineral compounds of transition elements. Magnetic particles may include: one or more magnetic crystals with and without nonmagnetic material. The nonmagnetic material can include synthetic or natural polymers (such as sepharose, dextran, dextrin, starch and the like.

The plasma kallikrein binding protein can also be labeled with an indicating group containing of the NMR active ^{19}F atom, or a plurality of such atoms inasmuch as (i) substantially all of naturally abundant fluorine atoms are the ^{19}F isotope and, thus, substantially all fluorine containing compounds are NMR active; (ii) many chemically active polyfluorinated compounds such as trifluoracetic anhydride are commercially available at relatively low cost; and (iii) many fluorinated compounds have been found medically acceptable for use in humans such as the perfluorinated polyethers utilized to carry oxygen as hemoglobin replacements. After permitting such time for incubation, a whole body MRI is carried out using an apparatus such as one of those described by Pykett, 1982, *Sci. Am.* 246:78 88 to locate and image tissues expressing plasma kallikrein.

The following examples provide further illustration and are not limiting.

EXAMPLES

Example 1

We have discovered several antibody inhibitors and binders of plasma kallikrein (pKal). The most potent of these have been further characterized and shown to have apparent inhibition constants ($K_{i,app}$)<10 nM, to be specific pKal inhibitors with respect to other tested serine proteases, and to not bind prekallikrein. Amino acid sequences of the CDRs for the inhibitors and the binders are shown in Tables 1 and 2, respectively.

TABLE 1

CDR Amino Acid Sequences, ELISA Signal, and Apparent Inhibition Constant of Antibody Inhibitors of PKal

Initial Name	Human pKal ELISA (T/B)	Human pKal (Ki, app nM)	LV-CDR1	LV-CDR2	LV-CDR3	HV-CDR1	HV-CDR2	HV-CDR3
M6-D09	39.9	5.9	RASQSIRNYLN	AASTLQS	QQLSGYPHT	FYYMV	VIYPSGGITVYAD SVKG	DKWAVMPPYYYYAMDV
M7-B04	4.1	54	TGTNSDVGNYNLVS	EVNKRPS	CSYAGNRNFYV	WYSMV	SISPSGGLTNYAD SVKG	HTAARPFYYYYMDV
M7-E07	45.7	36	SGDKLGDKYAC	QDSKRPS	QAWDSSTGV	WYLMI	YIYPSGGFTYYAD SVKG	TEGPLSWGYGMDV
M8-A09	5.4	105	SGDKLGNKYAY	QDNNRPS	QAWDSRTVV	TYFML	SIYPSGGNTVYAD SVKG	AASPVRNYYYYGMDV
M10-F10	39.2	<100 nM	RASQSISVYLN	GASNLQF	QQTFSLFT	FYNMN	SISPSGGETNYAD SVKG	GGGAYRNNWWGGFDI
M10-H05	42.2	18	RASQSVSSSYLA	GASSRAT	QQYGSSPFT	PYNMY	SIRPSGGGTVYAD SVKG	GFIAARWYYFDY
M12-D05	48.5	5.2	SGDQLGDKYVG	QDTKRPS	QAWDTSTAG	WYTMV	RIYPSGGWTKYAD SVKG	EGLLWFGENAFDI
M27-E05	41.3	16	SGDKLGDKYAC	QDSKRPS	QAWDSSTGV	WYLMI	YIYPSGGFTYYAD SVKG	TEGPLSWGYGMDV
M28-B11	33.3	5.5	SGDQLGDKYVG	QDTKRPS	QAWDTSTAG	WYTMV	RIYPSGGWTKYAD SVKG	EGLLWFGENAFDI
M29-D09	47.5	0.7	SGNKLGDKYVA	QDTKRPS	QAWDSSIVI	WYTMV	YIYPSGGATFYAD SVKG	GSYDYIWGFYSDH
M29-E09	28.8	11	SGDNLGNKYNS	QDTKRPS	QAWDGNVV	WYEMG	SIYSSGGGTMYAD SVKG	NPQYSGYDRSLSDGAFDI
M35-G04	11.1	2.9	RASQSVSSYLA	DASNRAT	QQRSNWPRGFT	YYHMS	VISPSGGSTKYAD SVKG	GGSSDYAWGSYRRPYYFDY

TABLE 1-continued

CDR Amino Acid Sequences, ELISA Signal, and Apparent Inhibition Constant of Antibody Inhibitors of PKal

Initial Name	Human pKal ELISA (T/B)	Human pKal (Ki, app nM)	LV-CDR1	LV-CDR2	LV-CDR3	HV-CDR1	HV-CDR2	HV-CDR3
M38-F02	33.5	14	SGEKLGDKYVS	EDSRRPS	QAWDSSTAI	YYMMV	YIYSSGGHTVYAD SVKG	DLFLYDFWSKGAFDI
M41-A11	28.0	13	SGDKLGDKYTS	QDIKRPS	QAWDSPNARV	HYRMS	SIYPSGGRTVYAD SVKG	DKFEWRLLFRGIGNDAFDI
M73-D06	4.0	<100 nM	SGSSSNIGSNTVS	NDHRRPS	SAWDDSLNGVV	RYEMY	SISSSGGPTAYAD SVKG	GTPKWELLLRSIYIENAFDI
M76-D01	11.2	<100 nM	RSSQSLSDDGNTYLD	TLSYRAS	MQGTHWPPT	FYAMH	GIVPSGGRTHYAD SVKG	DSSGSPNPLFDY
M110-C12	2.4	<100 nM	RSSLSLLHSNGYNYLD	LSSTRAS	MQPLETPPT	YYEMD	GISSSGGHTAYAD SVKG	ERRSSSRARYYYGMDV
M137-E12	4.5	79	SGNNSNFGSNTVT	SDSRRPS	AAWDDSLNGV	DYRMQ	VIVPSGGNTMYAD SVKG	GGPGSSIAARRAPTGYYGMDV
M142-H08	29.9	0.2	RASQPIDNYLN	AASRLQS	QQSYTVPYT	AYSMI	YIRPSGGRTTYAD SVKG	GGLLLWFRELKSNYFDY
M145-D01	6.2	1.1	RASQSVSSYLA	DASNRAT	QQRSNWPRGFT	YYHMS	VISPSGGSTKYAD SVKG	GGSSDYAWGSYRRPYYFDY
M145-D11	40.0	0.79	SGDKLGDKYTS	QDIKRPS	QAWDSPNARV	HYRMS	SIYPSGGRTVYAD SVKG	DKFEWRLLFRGIGNDAFDI
M146-E12	49.6	2.2	RASGDIGNALG	DASTLQS	LQGYNYPRT	RYIMH	SISPSGGLTSYAD SVKG	EFENAYHYYYYGMDV
M152-A12	19.	<100 nM	RASQSISSYLS	AASSLQS	QQSISIPRT	PYFMG	GIGPSGGSTTYAD SVKG	EGPPYSSGWYRGLRQYHFDY
M160-G12	38.3	17	RASQGISSYLA	AASTLQS	QQLNSYPLT	HYLMT	YISPSGGHTIYAD SVKG	VARGIAARSRTSYFDY
M161-C11	41.8	0.3	SGDKLGDKYVS	QDTKRPS	QAWDSSTYV	DYAMK	SISSSGGVTQYAD SVKG	EEDYSSSWYSRRFDYYYGMDV
M162-A04	11.4	4.8	RASQSISSWLA	KASTLES	QQYNTYWT	HYIMM	GIYSSGGITVYAD SVKG	RRTGIPRRDAFDI
X67-B03	nd	2.1	RASQPIDNYLN	AASRLQS	QQSYTVPYT	AYSMI	YIRPSGGRTTYAD SVKG	GGLLLWSRELKSNYFDY
X67-C03	nd	0.7	RASQPIDNYLN	AASRLQS	QQSYTVPYT	AYSMI	YIRPSGGRTTYAD SVKG	GGLLLWFMELKSNYFDY
X67-C09	nd	8.6	RASQPIDNYLN	AASRLQS	QQSYTVPYT	AYSMI	YIRPSGGRTTYAD SVKG	GGLLLWGRELKSNYFDY
X67-D03	nd	0.1	RASQPIDNYLN	AASRLQS	QQSYTVPYT	AYSMI	YIRPSGGRTTYAD SVKG	GGLLLWNRELKSNYFDY
X67-E04	nd	1.3	RASQPIDNYLN	AASRLQS	QQSYTVPYT	AYSMI	YIRPSGGRTTYAD SVKG	GGLLLWDRELKSNYFDY
X67-F01	nd	0.9	RASQPIDNYLN	AASRLQS	QQSYTVPYT	AYSMI	YIRPSGGRTTYAD SVKG	GGLLLWQRELKSNYFDY
X67-F10	nd	1.3	RASQPIDNYLN	AASRLQS	QQSYTVPYT	AYSMI	YIRPSGGRTTYAD SVKG	GGLLLWTRELKSNYFDY
X67-G04	nd	0.35	RASQPIDNYLN	AASRLQS	QQSYTVPYT	AYSMI	YIRPSGGRTTYAD SVKG	GGLLLWARELKSNYFDY
X67-H04	nd	3.6	RASQPIDNYLN	AASRLQS	QQSYTVPYT	AYSMI	YIRPSGGRTTYAD SVKG	GGLLLWERELKSNYFDY

TABLE 1-continued

CDR Amino Acid Sequences, ELISA Signal, and Apparent Inhibition Constant of Antibody Inhibitors of PKal

Initial Name	Human pKal ELISA (T/B)	Human pKal (Ki, app nM)	LV-CDR1	LV-CDR2	LV-CDR3	HV-CDR1	HV-CDR2	HV-CDR3
X81-B01	nd	0.2	RTSQFVNSNYLA	GASSRAT	QQSSRTPWT	HYLMT	YISPSGGHTIYAD SVKG	VARGIAARSRTSYFDY

Abbreviations used: "T/B" is the ELISA signal obtained using of the "target" (biotinylated plasma kallikrein) divided by the ELISA signal of the "background" (streptavidin); both of which were coated on microtiter plates. "nd" is not determined. The symbol "q" refers to the amber suppressible stop codon (TAG), which is translated as glutamine (Q) in strains of *E. coli* such as the TG1 cells that were used to express the Fab fragments.

Amino Acid Sequences of Light Chain (LC) and Heavy Chain (HC) Variable Domain of pKal Antibody Inhibitors are Shown Below.

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M6-D09                LC
QDIQMTQSPS SLSASVGDRV TITCRASQSI RNYLNWYQQK PGKAPNLLIY AASTLQSGVP    60
ARFSGSGSGT DFTLTISSLQ PEDFATYYCQ QLSGYPHTFG QGTKLEIK               108

M6-D09                HC
EVQLLESGGG LVQPGGSLRL SCAASGFTFS FYYMVWVRQA PGKGLEWVSV IYPSGGITVY    60
ADSVKGRFTI SRDNSKNTLY LQMNSLRAED TAVYYCARDK WAVMPPYYYY AMDVWGQGTT   120
VTVSSASTKG PSVFPLAPSS KS                                            142

M7-B04                LC
QSALTQPASV SGSPGQSITI SCTGTNSDVG NYNLVSWYQQ HPGEAPKLLI YEVNKRPSGV    60
SNRFSGSKSG NTASLTISGL QAEDEADYLC CSYAGNRNFY VFGAGTKVTV L            111

M7-B04                HC
EVQLLESGGG LVQPGGSLRL SCAASGFTFS WYSMVWVRQA PGKGLEWVSS ISPSGGLTNY    60
ADSVKGRFTI SRDNSKNTLY LQMNSLRAED TAVYYCARHT AARPFYYYYM DVWGKGTTVT   120
VSSASTKGPS VFPLAPSSKS                                               140

M7-E07                LC
QSELTQPPSV SVSPGQTASI TCSGDKLGDK YACWYQQKPG QSPVLVIYQD SKRPSGIPER    60
FSGSNSGNTA TLTISGTQAM DEADYYCQAW DSSTGVFGGG TKLTVL                  106

M7-E07                HC
EVQLLESGGG LVQPGGSLRL SCAASGFTFS WYLMIWVRQA PGKGLEWVSY IYPSGGFTYY    60
ADSVKGRFTI SRDNSKNTLY LQMNSLRAED MAVYYCARTE GPLSWGYGMD VWGQGTTVTV   120
SSASTKGPSV FPLAPSSKS                                                139

M8-A09                LC
QCELTQPPSE SVSPGQTANI TCSGDKLGNK YAYWYQQKPG QSPVLVIYQD NNRPSGIPER    60
FSGSNSGNTA TLTISGTQAI DEANYYCQAW DSRTVVFGGG TKLTVL                  106

M8-A09                HC
EVQLLESGGG LVQPGGSLRL SCAASGFTFS TYFMLWVRQA PGKGLEWVSS IYPSGGNTVY    60
ADSVKGRFTI SRDNSKNTLY LQMNSLRAED TAVYYCARAA SPVRNYYYYG MDVWGQGTTV   120
TVSSASTKGP SVFPLAPSSK S                                             141

M10-F10                LC
QDIQMTQSPS SLSASVGDRV TITCRASQSI SVYLNWYQHK PGKAPKLLIY GASNLQFGVP    60
SRFSGSGYGT DFTLTISSLQ PEDFATYHCQ QTFSLFTFGG GTKVEIK                 107

M10-F10                HC
EVQLLESGGG LVQPGGSLRL SCAASGFTFS FYNMNWVRQA PGKGLEWVSS ISPSGGETNY    60
ADSVKGRFTI SRDNSKNTLY LQMNSLRAED TAVYYCARGG GAYRNNWWGG FDIWGLGTMV   120
TVSSASTKGP SVFPLAPSSK S                                             141

M10-H05                LC
QDIQMTQSPG TLSLSPGERA TLSCRASQSV SSSYLAWYQQ KPGQAPRLLI YGASSRATGI    60
PDRFSGSGSG TDFTLTISRL EPEDFAVYYC QQYGSSPFTF GPGTKVDIK               109

M10-H05                HC
EVQLLESGGG LVQPGGSLRL SCAASGFTFS PYNMYWVRQA PGKGLEWVSS IRPSGGGTVY    60
ADSVKGRFTI SRDNSKNTLY LQMNSLRAED TAVYYCAGGF IAARWYYFDY WGQGTLVTVS   120
SASTKGPSVF PLAPSSKS                                                 138

M12-D05                LC
QSVLTQPPSV SVSPGQTATI TCSGDQLGDK YVGWYQQKPG QSPILVIYQD TKRPSGIPER    60
FSGSNSGNTA TLTISGTHTV DEAHYYCQAW DTSTAGFGGG TKLTVL                  106
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M12-D05               HC
EVQLLESGGG LVQPGGSLRL SCAASGFTFS WYTMVWVRQA PGKGLEWVSR IYPSGGWTKY    60
ADSVKGRFTI SRDNSKNTLY LQMNSLRAED TATYYCAREG LLWFGENAFDIWGQGTMVTV    120
SSASTKGPSV FPLAPSSKS                                                 139

M27-E05               LC
QSELTQPPSV SVSPGQTASI TCSGDKLGDK YACWYQQKPG QSPVLVIYQD SKRPSGIPER    60
FSGSNSGNTA TLTISGTQAM DEADYYCQAW DSSTGVFGGG TKLTVL                   106

M27-E05               HC
EVQLLESGGG LVQPGGSLRL SCAASGFTFS WYLMIWVRQA PGKGLEWVSY IYPSGGFTYY    60
ADSVKGRFTI SRDNSKNTLY LQMNSLRAED MAVYYCARTE GPLSWGYGMD VWGQGTTVTV   120
SSASTKGPSV FPLAPSSKS                                                 139

M28-B11               LC
QSVLTQPPSV SVSPGQTATI TCSGDQLGDK YVGWYQQKPG QSPILVIYQD TKRPSGIPER    60
FSGSNSGNTA TLTISGTHTV DEAHYYCQAW DTSTAGFGGG TKLTVL                   106

M28-B11               HC
EVQLLESGGG LVQPGGSLRL SCAASGFTFS WYTMVWVRQA PGKGLEWVSR IYPSGGWTKY    60
ADSVKGRFTI SRDNSKNTLY LQMNSLRAED TATYYCAREG LLWFGENAFD IWGQGTMVTV   120
SSASTKGPSV FPLAPSSKS                                                 139

M29-D09               LC
QSALTQPPTV SVSPGQTARI TCSGNKLGDK YVAWYQQKPG QSPMLVIYQD TKRPSRVSER    60
FSGSNSANTA TLSISGTQAL DEADYYCQAW DSSIVIFGGG TRLTVL                   106

M29-D09               HC
EVQLLESGGG LVQPGGSLRL SCAASGFTFS WYTMVWVRQA PGKGLEWVSY IYPSGGATFY    60
ADSVKGRFTI SRDNSKNTLY LQMNSLRAED TAVYYCAMGS YDYIWGFYSD HWGQGTLVTV   120
SSASTKGPSV FPLAPSSKS                                                 139

M29-E09               LC
QYELTQPPSV SVSPGQTATI TCSGDNLGNK YNSWYQQKPG QSPLLVIYQD TKRPSAIPER    60
FSGSNSGNTA TLTISGTQAM DEADYYCQAW DGNVVFGGGT KLTVL                    105

M29-E09               HC
EVQLLESGGG LVQPGGSLRL SCAASGFTFS WYEMGWVRQA PGKGLEWVSS IYSSGGGTMY    60
ADSVKGRFTI SRDNSKNTLY LQMNSLRAED TAVYYCARNP QYSGYDRSLS DGAFDIWGQG   120
TMVTVSSAST KGPSVFPLAP SSKS                                           144

M35-G04               LC
QDIQMTQSPA TLSLSPGERA TLSCRASQSV SSYLAWYQQK PGQAPRLLIY DASNRATGIP    60
ARFSGSGSGT DFTLTISSLE PEDFAVYYCQ QRSNWPRGFT FGPGTKVDIK               110

M35-G04               HC
EVQLLESGGG LVQPGGSLRL SCAASGFTFS YYHMSWVRQA PGKGLEWVSV ISPSGGSTKY    60
ADSVKGRFTI SRDNSKNTLY LQMNSLRAED TAVYYCARGG SSDYAWGSYR RPYYFDYWGQ   120
GTLVTVSSAS TKGPSVFPLA PSSKS                                          145

M38-F02               LC
QSVLTQPPSV SVSPGQTASI TCSGEKLGDK YVSWYQQKPG QSPSLVICED SRRPSGIPER    60
FSGSNSGNTA TLTISGAQPM DEADYYCQAW DSSTAIFGPG TKVTVL                   106

M38-F02               HC
EVQLLESGGG LVQPGGSLRL SCAASGFTFS YYMMVWVRQA PGKGLEWVSY IYSSGGHTVY    60
ADSVKGRFTI SRDNSKNTLY LQMNSLRAED TAVYYCARDL FLYDFWSKGA FDIWGQGTMV   120
TVSSASTKGP SVFPLAPSSK S                                              141

M41-A11               LC
QSVLTQPPSV SVSPGQTASI TCSGDKLGDK YTSWYQQRPG QSPVLVIYQD IKRPSGIPER    60
FSGSNSGNTA TLTISGTQAM DEADYYCQAW DSPNARVFGS GTKVTVL                  107

M41-A11               HC
EVQLLESGGG LVQPGGSLRL SCAASGFTFS HYRMSWVRQA PGKGLEWVSS IYPSGGRTVY    60
ADSVKGRFTI SRDNSKNTLY LQMNSLRAED TAVYYCAKDK FEWRLLFRGI GNDAFDIWGQ   120
GTMVTVSSAS TKGPSVFPLA PSSKS                                          145

M73-D06               LC
QSELTQPPSA SETPGQRVTI SCSGSSSNIG SNTVSWFQQL PGSAPRLLIY NDHRRPSGVP    60
DRFSGSKSGT SASLVISGLQ SQDEADYYCS AWDDSLNGVV FGGGTKLTVL               110

M73-D06               HC
EVQLLESGGG LVQPGGSLRL SCAASGFTFS RYEMYWVRQA PGKGLEWVSS ISSSGGPTAY    60
ADSVKGRFTI SRDNSKNTLY LQMNSLRAED TAMYYCAKGT PKWELLLRSI YIENAFDIWG   120
QGTMVTVSSA STKGPSVFPL APSSKS                                         146

M76-D01               LC
QDIVMTQTPP SLPVNPGEPA SISCRSSQSL SDDGNTYLDW YLQRPGQSPQ LLIHTLSYRA    60
SGVPDRFSGS GSGTDFTLKI SRVEAEDVGV YYCMQGTHWP PTFGQGTKVE IK            112
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M76-D01              HC
EVQLLESGGG LVQPGGSLRL SCAASGFTFS FYAMHWVRQA PGKGLEWVSG IVPSGGRTHY    60
ADSVKGRFTI SRDNSKNTLY LQMNSLRAED TAVYYCATDS SGSPNPLFDY WGQGTLVTVS   120
SASTKGPSVF PLAPSPKS                                                  138

M110-C12              LC
QDIQMTQSPL SLSVTPGEPA SISCRSSLSL LHSNGYNYLD WYVQRPGQSP QLLMYLSSTR    60
ASGVPDRFSG SGSGTDFTLE ISRVEAEDVG VYYCMQPLET PPTFGGGTKV EIK          113

M110-C12              HC
EVQLLESGGG LVQPGGSLRL SCAASGFTFS YYEMDWVRQA PGKGLEWVSG ISSSGGHTAY    60
ADSVKGRFTI SRDNSKNTLY LQMNSLRAED TATYYCARER RSSSRARYYY GMDVWGQGTT   120
VTVSSASTKG PSVFPLAPSS KS                                             142

M137-E12              LC
QSVLIQPPSV SGIPGQRVTI SCSGNNSNFG SNTVTWYQQL PGTAPKLLIY SDSRRPSGVP    60
DRFSGSRSDT SASLAISGLQ SEDEAEYHCA AWDDSLNGVF GGGTKLTVL               109

M137-E12              HC
EVQLLESGGG LVQPGGSLRL SCAASGFTFS DYRMQWVRQA PGKGLEWVSV IVPSGGNTMY    60
ADSVKGRFTI SRDNSKNTLY LQMNSLRAED TAVYYCARGG PGSSIAARRA PTGYYGMDVW   120
GQGTTVTVSS ASTKGPSVFP LAPSSKS                                        147

M142-H08              LC
QDIQMTQSPS SLSAFVGDRV TITCRASQPI DNYLNWYHQK PGKAPKLLIY AASRLQSGVP    60
SRLSGSGFGT DFTLTISSLQ PEDFGNYYCQ QSYTVPYTFG GGTKVEIR                108

M142-H08              HC
EVQLLESGGG LVQPGGSLRL SCAASGFTFS AYSMIWVRQA PGKGLEWVSY IRPSGGRTTY    60
ADSVKGRFTI SRDNSKNTLY LQMNSLRAED TAVYYCARGG LLLWFRELKS NYFDYWGQGT   120
LVTVSSASTK GPSVFPLAPS SKS                                            143

M145-D01              LC
QDIQMTQSPA TLSLSPGERA TLSCRASQSV SSYLAWYQQK PGQAPRLLIY DASNRATGIP    60
ARFSGSGSGT DFTLTISSLE PEDFAVYYCQ QRSNWPRGFT FGPGTKVDIK              110

M145-D01              HC
EVQLLESGGG LVQPGGSLRL SCAASGFTFS YYHMSWVRQA PGKGLEWVSV ISPSGGSTKY    60
ADSVKGRFTI SRDNSKNTLY LQMNSLRAED TAVYYCARGG SSDYAWGSYR RPYYFDYWGQ   120
GTLVTVSSAS TKGPSVFPLA PSSKS                                          145

M145-D11              LC
QSVLTQPPSV SVSPGQTASI TCSGDKLGDK YTSWYQQRPG QSPVLVIYQD IKRPSGIPER    60
FSGSNSGNTA TLTISGTQAM DEADYYCQAW DSPNARVFGS GTKVTVL                 107

M145-D11              HC
EVQLLESGGG LVQPGGSLRL SCAASGFTFS HYRMSWVRQA PGKGLEWVSS IYPSGGRTVY    60
ADSVKGRFTI SRDNSKNTLY LQMNSLRAED TAVYYCAKDK FEWRLLFRGI GNDAFDIWGQ   120
GTMVTVSSAS TKGPSVFPLA PSSKS                                          145

M146-E12              LC
QDIQMTQSPS SLSASVGDRV TITCRASGDI GNALGWYQQK PGKAPRLLIS DASTLQSGVP    60
LRFSGSGSGT EFTLTISSLQ PEDFATYYCL QGYNYPRTFG QGTKLEIR                108

M146-E12              HC
EVQLLESGGG LVQPGGSLRL SCAASGFTFS RYIMHWVRQA PGKGLEWVSS ISPSGGLTSY    60
ADSVKGRFTI SRDNSKNTLY LQMNSLRAED TAVYYCAREF ENAYHYYYYG MDVWGQGTTV   120
TVSSASTKGP SVFPLAPSSK S                                              141

M152-A12              LC
QDIQMTQSPS SLSASVGDRV TITCRASQSI SSYLSWYQQR PGKAPNLLIY AASSLQSGVP    60
SRFSGSGSGT DFTLTISSLQ PEDFATYYCQ QSISIPRTFG QGTKVEVK                108

M152-A12              HC
EVQLLESGGG LVQPGGSLRL SCAASGFTFS PYFMGWVRQA PGKGLEWVSG IGPSGGSTTY    60
ADSVKGRFTI SRDNSKNTLY LQMNSLRAED TAVYYCAREG PPYSSGWYRG LRQYHFDYWG   120
QGTLVTVSSA STKGPSVFPL APSSKS                                         146

M160-G12              LC
QDIQMTQSPS FLSASVGDRV TITCRASQGI SSYLAWYQQK PGKAPKLLIY AASTLQSGVP    60
SRFSGSGSGT EFTLTISSLQ PEDFATYYCQ QLNSYPLTFG GGTKVEIK                108

M160-G12              HC
EVQLLESGGG LVQPGGSLRL SCAASGFTFS HYLMTWVRQA PGKGLEWVSY ISPSGGHTIY    60
ADSVKGRFTI SRDNSKNTLY LQMNSLRAED TAVYYCARVA RGIAARSRTS YFDYWGQGTL   120
VTVSSASTKG PSVFPLAPSS KS                                             142

M161-C11              LC
QSALTQPPSV SVSPGQTASI TCSGDKLGDK YVSWYQQRPG QSPVLVIYQD TKRPSGIPER    60
FSGSNSGNTA TLTISGTQAV DEADYYCQAW DSSTYVFGGG TKVTVL                  106
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M161-C11                 HC
EVQLLESGGG LVQPGGSLRL SCAASGFTFS DYAMKWVRQA PGKGLEWVSS ISSSGGVTQY    60
ADSVKGRFTI SRDNSKNTLY LQMNSLRAED TAVYYCAREE DYSSSWYSRR FDYYYGMDVW   120
GQGTTVTVSS ASTKGPSVFP LAPSSKS                                       147

M162-A04                 LC
QDIQMTQSPS TLSASVGDRV TITCRASQSI SSWLAWYQQK PGKAPNLLIY KASTLESGVP    60
SRFSGSGSGT EFTLTISSLQ PDDFATYYCQ QYNTYWTFGQ GTKVEIK                 107

M162-A04                 HC
EVQLLESGGG LVQPGGSLRL SCAASGFTFS HYIMMWVRQA PGKGLEWVSG IYSSGGITVY    60
ADSVKGRFTI SRDNSKNTLY LQMNSLRAED TAVYYCAYRR TGIPRRDAFD IWGQGTMVTV   120
SSASTKGPSV FPLAPSSKS                                                139

X67-B03 LC
QDIQMTQSPS SLSAFVGDRV TITCRASQPI DNYLNWYHQK PGKAPKLLIY AASRLQSGVP    60
SRLSGSGFGT DFTLTISSLQ PEDFGNYYCQ QSYTVPYTFG GGTKVEIR                108

X67-B03 HC
EVQLLESGGG LVQPGGSLRL SCAASGFTFS AYSMIWVRQA PGKGLEWVSY IRPSGGRTTY    60
ADSVKGRFTI SRDNSKNTLY LQMNSLRAED TAVYYCARGG LLLWSRELKS NYFDYWGQGT   120
LVTVSSASTK GPSVFPLAPS SKS                                           143

X67-C03 LC
QDIQMTQSPS SLSAFVGDRV TITCRASQPI DNYLNWYHQK PGKAPKLLIY AASRLQSGVP    60
SRLSGSGFGT DFTLTISSLQ PEDFGNYYCQ QSYTVPYTFG GGTKVEIR                108

X67-C03 HC
EVQLLESGGG LVQPGGSLRL SCAASGFTFS AYSMIWVRQA PGKGLEWVSY IRPSGGRTTY    60
ADSVKGRFTI SRDNSKNTLY LQMNSLRAED TAVYYCARGG LLLWMRELKS NYFDYWGQGT   120
LVTVSSASTK GPSVFPLAPS SKS                                           143

X67-C09 LC
QDIQMTQSPS SLSAFVGDRV TITCRASQPI DNYLNWYHQK PGKAPKLLIY AASRLQSGVP    60
SRLSGSGFGT DFTLTISSLQ PEDFGNYYCQ QSYTVPYTFG GGTKVEIR                108

X67-C09 HC
EVQLLESGGG LVQPGGSLRL SCAASGFTFS AYSMIWVRQA PGKGLEWVSY IRPSGGRTTY    60
ADSVKGRFTI SRDNSKNTLY LQMNSLRAED TAVYYCARGG LLLWGRELKS NYFDYWGQGT   120
LVTVSSASTK GPSVFPLAPS SKS                                           143

X67-D03 LC
QDIQMTQSPS SLSAFVGDRV TITCRASQPI DNYLNWYHQK PGKAPKLLIY AASRLQSGVP    60
SRLSGSGFGT DFTLTISSLQ PEDFGNYYCQ QSYTVPYTFG GGTKVEIR                108

X67-D03 HC
EVQLLESGGG LVQPGGSLRL SCAASGFTFS AYSMIWVRQA PGKGLEWVSY IRPSGGRTTY    60
ADSVKGRFTI SRDNSKNTLY LQMNSLRAED TAVYYCARGG LLLWNRELKS NYFDYWGQGT   120
LVTVSSASTK GPSVFPLAPS SKS                                           143

X67-E04 LC
QDIQMTQSPS SLSAFVGDRV TITCRASQPI DNYLNWYHQK PGKAPKLLIY AASRLQSGVP    60
SRLSGSGFGT DFTLTISSLQ PEDFGNYYCQ QSYTVPYTFG GGTKVEIR                108

X67-E04 HC
EVQLLESGGG LVQPGGSLRL SCAASGFTFS AYSMIWVRQA PGKGLEWVSY IRPSGGRTTY    60
ADSVKGRFTI SRDNSKNTLY LQMNSLRAED TAVYYCARGG LLLWDRELKS NYFDYWGQGT   120
LVTVSSASTK GPSVFPLAPS SKS                                           143

X67-F01 LC
QDIQMTQSPS SLSAFVGDRV TITCRASQPI DNYLNWYHQK PGKAPKLLIY AASRLQSGVP    60
SRLSGSGFGT DFTLTISSLQ PEDFGNYYCQ QSYTVPYTFG GGTKVEIR                108

X67-F01 HC
EVQLLESGGG LVQPGGSLRL SCAASGFTFS AYSMIWVRQA PGKGLEWVSY IRPSGGRTTY    60
ADSVKGRFTI SRDNSKNTLY LQMNSLRAED TAVYYCARGG LLLWQRELKS NYFDYWGQGT   120
LVTVSSASTK GPSVFPLAPS SKS                                           143

X67-F10 LC
QDIQMTQSPS SLSAFVGDRV TITCRASQPI DNYLNWYHQK PGKAPKLLIY AASRLQSGVP    60
SRLSGSGFGT DFTLTISSLQ PEDFGNYYCQ QSYTVPYTFG GGTKVEIR                108

X67-F10 HC
EVQLLESGGG LVQPGGSLRL SCAASGFTFS AYSMIWVRQA PGKGLEWVSY IRPSGGRTTY    60
ADSVKGRFTI SRDNSKNTLY LQMNSLRAED TAVYYCARGG LLLWTRELKS NYFDYWGQGT   120
LVTVSSASTK GPSVFPLAPS SKS                                           143

X67-G04 LC
QDIQMTQSPS SLSAFVGDRV TITCRASQPI DNYLNWYHQK PGKAPKLLIY AASRLQSGVP    60
SRLSGSGFGT DFTLTISSLQ PEDFGNYYCQ QSYTVPYTFG GGTKVEIR                108
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X67-G04 HC
EVQLLESGGG LVQPGGSLRL SCAASGFTFS AYSMIWVRQA PGKGLEWVSY IRPSGGRTTY 60
ADSVKGRFTI SRDNSKNTLY LQMNSLRAED TAVYYCARGG LLLWARELKS NYFDYWGQGT 120
LVTVSSASTK GPSVFPLAPS SKS 143

X67-H04 LC
QDIQMTQSPS SLSAFVGDRV TITCRASQPI DNYLNWYHQK PGKAPKLLIY AASRLQSGVP 60
SRLSGSGFGT DFTLTISSLQ PEDFGNYYCQ QSYTVPYTFG GGTKVEIR 108

X67-H04 HC
EVQLLESGGG LVQPGGSLRL SCAASGFTFS AYSMIWVRQA PGKGLEWVSY IRPSGGRTTY 60
ADSVKGRFTI SRDNSKNTLY LQMNSLRAED TAVYYCARGG LLLWERELKS NYFDYWGQGT 120
LVTVSSASTK GPSVFPLAPS SKS 143

Note: X81-B01 is a germilined IgG derived from X63-G06 which is shown in Table 7.

TABLE 2

CDR Amino Acid Sequences and ELISA Signal of Antibody Binders of PKal

Initial Name	Human pKal ELISA (T/B)	LV-CDR1	LV-CDR2	LV-CDR3	HV-CDR1	HV-CDR2	HV-CDR3
M6-A06	11.7	RASQSISMYLN	GTSSLQS	QQSYSAPWT	LYQMT	GIWPSGGFTDYADSVKG	VSTAVADNDY
M6-A08	23.4	RASQRISFYLN	GASSLQS	QQTFSTPNT	PYPMQ	SISSSGGMTEYADSVKG	DDYGGKGGAFDI
M6-D03	15.5	RASQSISSYLN	AASSLQS	QQSYSTLWT	KYFMG	VIGSSGGWTSYADSVKG	VSTAVADNDY
M6-D08	16	RASQSISSYLN	GASSLQS	QQSYTRWT	RYHMV	SISPSGGWTNYADSVKG	EMATIAGQFDP
M6-G05	18.5	RASQSISTYLN	NAFSMER	QQSYTTPTT	RYRMV	SIYPSGGMTAYADSVKG	DAVGIGDAFDI
M8-C04	44.7	SGDKLGDKYTS	QDSKRPS	QAWDSSTV	YYPMQ	YIYPSGGLTSYADSVKG	LFYGSGSVGFEY
M8-D05	11.9	RASQDISSWLV	DASNLQS	QQADGFPLT	LYNMN	SISPSGGFTDYADSVKG	DLDLGILDY
M8-E06	8.8	RASQSISSYLN	AASSLQS	QQSYSTLMYT	HYFMT	SIVPSGGMTQYADSVKG	DSYSSSWFDI
M8-G09	28.2	RASQGVSYYLA	GASSLQS	QQYNTYPPT	LYEML	VIYPSGGYTDYADSVKG	SFSGFGEIDY
M8-H04	3.3	RASQYISTYLN	GTSSLQS	QQSFTTPFT	GYWMG	SISSSGGWTQYADSVKG	DDEIAAGGAFDI
M9-A03	14.4	RASQNIDIYLN	GAYNLQS	QQSYGTPV	GYFMM	SIYSSGGYTDYADSVKG	EVAGTYAFDI
M9-A08	5.5	RASQRISTYLN	GASSLQS	QQSYNTPRT	AYEMW	YIGSSGGSTSYADSVKG	GNSSSFDAFDI
M9-C08	10.9	RASQSISIYVN	AASSLQR	QQSFSTPLT	HYGMV	YIVPSGGLTYYADSVKG	VDYTGDGLGY
M9-C10	7.8	RASQGISSYLN	GASSLQS	QESYSTLFT	LYPMQ	SIGSSGGMTFYADSVKG	EVGAAGFAFDI
M9-D08	35.9	RASRTISFYLN	GGSSLHS	QQSFSSPWT	WYKMM	SIYPSGGWTNYADSVKG	GSPWGDDAFDI
M9-E04	18.8	RASQSISGYLN	AASNLQT	QQSHTPPKT	EYDMM	SIGSSGGMTYYADSVKG	DQVAAAAIDY
M9-F08	10.9	RASQSISSYLN	AASSLQS	QQSYSTPPYT	PYAMT	VIYPSGGFTDYADSVKG	ASGSYLDAFDI
M9-F09	7	RASQSISSYLN	AASSLQS	QQTYTTPWT	SYPMG	RISSSGGMTIYADSVKG	DDWNVGMDV
M9-F10	8.4	RASQSINTYLN	AASTLES	QQSYSTPYT	DYDME	SISPSGGSTIYADSVKG	QGLLTAFDI
M9-G08	4.8	RASQSISSYLN	AASSLQS	QQSYSTPIT	YYTML	SIYPSGGFTMYADSVKG	VDTAMAMIDY
M9-H02	3.5	RASRSIATYLN	GASTLQS	QQSFSDPYT	AYMMI	VIYPSGGVTMYADSVKG	GTVGASDAFDI
M9-H03	4.4	SGDKLGNRYTS	QDNKRPS	QALDSNTYV	WYSMG	YIVPSGGYTMYADSVKG	DPGVSYYYYGMDV
M9-H04	16.1	RASQSISSYLN	AASSLQS	QQSYSTPPT	AYTMW	SIWPSGGSTFYADSVKG	TYDSSAGEVDY
M10-A03	33.7	RASQRISFYLN	GASSLQS	QQTFSTPNT	PYPMQ	SISSSGGMTEYADSVKG	DDYGGKGGAFDI
M10-A12	20.8	RASRDISVYLN	GASSLQS	QQSYSIPFT	LYLMH	SIYSSGGFTTYADSVKG	DTDYGMDV
M10-B09	14.1	RASQSISTYLN	GASSLQS	QQSFSTPWT	WYEMS	RIWPSGGVTMYADSVKG	TSITTVGMDV
M10-C11	5.3	RASQSISIYLN	AASTLQS	QQSHSIPPT	MYPMM	YISPSGGMTDYADSVKG	VAGSSDAFDI

TABLE 2-continued

CDR Amino Acid Sequences and ELISA Signal of Antibody Binders of PKal

Initial Name	Human pKal ELISA (T/B)	LV-CDR1	LV-CDR2	LV-CDR3	HV-CDR1	HV-CDR2	HV-CDR3
M10-D11	6.4	RSSQSLLHSNGYNYLD	LGSNRAS	MQALQTPLT	AYPMN	RISSSGGNTSYADSVKG	GYLGY
M10-E06	32.8	RASQSISTYLN	GASSLQS	QQSYSDPYT	LYRMF	SIWSSGGPTMYADSVKG	EYPSTYYFDY
M10-F09	4.8	RASQTIDDDLI	AASSLQS	QQSYNIPRT	NYDMM	YISPSGGFTRYADSVKG	DIYYYNWGPSHYFDS
M10-G09	7.1	RASQSISGYIN	AASSLQS	QQYVSYPFT	QYGMQ	SIRSSGGATRYADSVKG	DGYYDSSGYPDY
M11-A10	25	RASQSIDTYLN	DASNL	QHYLYAPYS	NYWMM	GIGSSGGFTSYADSVKG	GSYSDYGVFES
M11-E01	11.7	RASQSISSYLN	AASSLQS	QQSYSTPPT	TYEMY	GIGSSGGMTMYADSVKG	EQPGIAALQF
M11-E04	43.2	RASQSISIYLT	GAATLQT	QQTFSLPRT	MYHMN	GIVSSGGVTFYADSVKG	ITTVTTGGAFDI
M11-E05	41.4	RTSQTINNYLN	ATHTLES	QQSFAFPYT	WYTMG	WIYFGGLTTYADSVKG	LGGPLDAFDI
M11-E06	12.6	RASRGIGTYLN	AASSLET	QESFTNVYN	QYAMH	SIYPSGGFTLYADSVKG	GGWLAGGELLN
M11-G09	23.6	RTSQGINHYLN	AASELQT	QQTYTSPYT	LYNMT	YIYPSGGGTHYADSVKG	DTGFWSADAFDI
M11-G12	4.9	RASQTISVYVN	GASSLQS	QQSYSIPFT	QYPMN	SISSSGGFTTYADSVKG	EEQQGGFDY
M12-A08	40.4	RASQSISRYLN	AASTLET	QQSYSTPYT	WYYMG	WIVSSGGLTLYADSVKG	TTVTTGDAFDI
M12-B04	18	RASQGIRNDLG	AASILQS	LQDYEYPLT	LYSMY	RIRPSGGGTVYADSVKG	DPLYSSGDV
M12-C09	7	RASQSIGIYLN	GASSLQS	QHSYSTPFT	SYAMV	SIGSSGGFTLYADSVKG	MNLGGGDAFDI
M12-C10	8.3	SGDKLGEKYVS	QDNKRPS	QAWDSYTVV	DYEMH	GISPSGGKTQYADSVKG	DLKWGGRGSPDWYFDL
M12-D10	9.9	RASQSISSYLN	AASSLQS	QQSYSTPPT	NYPMD	SISSSGGWTNYADSVKG	DTSGSYLGFDY
M12-E06	48	RASQSISTYLN	GAFSLQS	QQSHSTPPT	QYKML	GIGPSGGLTAYADSVKG	APWFGELGMDV
M27-A10	3.2	RASQSISAYLN	YGVGSLQS	QQGYTTPVT	WYRMD	SIWPSGGLTSYADSVKG	GWAPGGDAFDI
M27-B01	33.1	RASQSISSYLN	AASSLQS	QQSYSTPYT	DYTMW	SISSSGGITFYADSVKG	SADTAMGGAFDI
M27-B12	2.3	SGDKLGDEYAA	QDRKRPS	QAWGKRNVV	WYQMM	SISPSGGITEYADSVKG	DRSSGWYYYGMDV
M27-E03	35.9	RASQSISSYLN	AASSLQS	QQSYSTPRT	SYMMH	GIYPSGGWTDYADSVKG	LVAGLDAFDI
M27-F04	10.5	RASQSISSYLN	AASSLQS	QQSYSTPPT	WYPMT	SIGPSGGQTIYADSVKG	EYGDYGGGFDP
M27-F11	10	RASQGISSYLA	AASSLQS	QQSYNTLRT	SYHMM	SIYPSGGATMYADSVKG	DGYHYGDYTYFQH
M27-G01	31.4	RASQSISTYLN	GASSLQS	QQSYSDPYT	LYRMF	SIWSSGGPTMYADSVKG	EYPSTYYFDY
M27-G04	4.1	RASQRISYYLT	AASSLES	QQAFSTPFT	AYYMV	YISPSGGQTQYADSVKG	EAISSSSFDY
M27-G09	2.2	RTRQSISNYLN	AASSLQS	QQSYDIPFT	EYDMA	YIVSSGGFTSYADSVKG	WAGWIAAADY
M27-H10	12.4	RASQSISNYLN	AASSLQS	QQSYSTPQT	AYQMA	VIYSSGGYTDYADSVKG	HNWNDGAFDI
M28-A01	19	RASQSISSYLN	AASSLQS	QQSYSTLT	WYAMH	GIYSSGGYTKYADSVKG	DLSNGDDVFDI
M28-C03	2.2	RASQSINFYLN	VASSLES	LQSYSAPYT	YYQMG	SIYPSGGMTDYADSVKG	GSPWGDDAFDI
M28-D02	3.7	RTSRRIGTYLN	GASSLQS	QQSFSSPWT	WYPMQ	YIYPSGGGTDYADSVKG	SSGWLGDAFDI
M28-D12	41.6	RASQSIATYLN	AASSLQS	QQSYSTRET	WYTMH	VIYPSGGPTSYADSVKG	DGSGSYLGFDY
M28-E01	41	RASQSISSYLN	AASSLQS	QQTYTTPWT	SYPMG	RISSSGGMTIYADSVKG	DDWNVGMDV
M28-E11	29.3	RASQDISNWLA	AASSLQT	QQSYSLPWT	LYDMT	GISSSGGVTIYADSVKG	TYYYDSSGYADAFDI
M28-F01	1.5	RASQSINTYLN	AASTLES	QQSYSTPPT	VYLMH	GISPSGGYTQYADSVKG	PGGLDAFDI
M28-F05	31.4	RASQSISSYLN	AASSLQS	QQSYSTPLT	RYIMW	GIYSSGGYTQYADSVKG	ELEGLGGFDY
M28-F07	33	RASQGISSWLA	ATSGLQS	QQAKSFPLT	DYTMY	SIVPSGGHTLYADSVKG	DHLSSWYGGFFDY

TABLE 2-continued

CDR Amino Acid Sequences and ELISA Signal of Antibody Binders of PKal

Initial Name	Human pKal ELISA (T/B)	LV-CDR1	LV-CDR2	LV-CDR3	HV-CDR1	HV-CDR2	HV-CDR3
M29-C07	5.2	RASQSISSYLN	AASSLQS	QQSYSTRYT	GYDMM	VISSSGGNTAYADSVKG	ESSGLYYFDY
M29-D10	23.6	RASQSITIYLN	GASNLHS	QQSYDTPLT	WYPMY	SIGSSGGPTPYADSVKG	WADYGGSLDY
M29-E02	2	SGSSSNIGNNAVS	YDDLLPS	AAWDDSLNGFV	RYPMM	VIYPSGGDTFYADSVKG	GDDYLWEAAVY
M29-G08	40.4	RASQNIGNDVA	HASTRAY	QQFYDWPAHT	YYHMW	GISPSGGFTFYADSVKG	DYYYDSSGYSPLGY
M29-G10	16.4	RASQSISIYLN	GASQLES	QQSYNVPYT	FYKMI	SISSSGGSTQYADSVKG	DRVDLGYLDY
M74-A07	8.6	RTSQNINTYLN	GVSSLHR	QQSYSSPWT	QYLMM	SIYPSGGYTSYADSVKG	VSTAVADNDY
M76-F02	6.4	RASQTIDNYLH	DASSLQS	QQSYDTPQYT	LYDMN	GISPSGGQTMYADSVKG	QPMISAFDI
M76-G02	10.3	RASQSISSYLN	AASSLQS	QQSYSTPPWT	LYAMW	YISSSGGFTSYADSVKG	YRVGVAATDY
M76-G06	11.8	RASQSISTYLN	AASSLQS	QQSYSTPHT	GYIMH	WIYPSGGWTEYADSVKG	DAPGVGAIDY
M76-H02	13.4	RASQDISVYLN	GGASLQS	QQSYSLPFT	MYWMQ	YIYPSGGPTKYADSVKG	PSGSYGDAFDI
M77-C07	16.1	RASQNISSYLN	AASSLQS	QQSYSTPRT	LYIMG	GIYPSGGFTMYADSVKG	ESSGVAAPDY
M77-H04	7.6	RSSQSLLHSRGYNYLD	LGSNRAS	MQALQRRT	YYTMI	GIRSSGGGTRYADSVKG	DGSRYSYGSIYYYYGMDA

Abbreviations used: "T/B" is the ELISA signal obtained using of the target (biotinylated plasma kallikrein) divided by the ELISA signal of the "background" (streptavidin); both of which were coated on microtiter plates. "nd" is not determined. The symbol "q" refers to the amber suppressible stop codon (TAG), which is translated as glutamine (Q) in strains of *E. coli* such as the
TG1 cells that were used to express the Fab fragments.

Amino Acid Sequences of Light Chain (LC) and Heavy Chain (HC) Variable Domain of pKal Antibody Binders are Shown Below.

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M6-A06                  LC
QDIQMTQSPS SLSASVGDSV TISCRASQSI SMYLNWYQHK PGKAPKLLIY GTSSLQSGVP   60
SRFSGSGPGG TDFTLTISSL QPEDFATYYC QQSYSAPWTF GQGTKVEIK              109

M6-A06                  HC
EVQLLESGGG LVQPGGSLRL SCAASGFTFS LYQMTWVRQA PGKGLEWVSG IWPSGGFTDY   60
ADSVKGRFTI SRDNSKNTLY LQMNSLRAED TAVYYCARVS TAVADNDYWG QGTLVTVSSA  120
STKGPSVFPL APSSKS                                                  136

M6-A08                  LC
QDIQMTQSPS SLSASVGDRV TITCRASQRI SFYLNWFQQK PGKAPNLLIY GASSLQSGVP   60
SRFSGSGSGT DFTLTISSLQ PKDFGTYYCQ QTFSTPNTFG QGTKLEIK               108

M6-A08                  HC
EVQLLESGGG LVQPGGSLRL SCAASGFTFS PYPMQWVRQA PGKGLEWVSS ISSSGGMTEY   60
ADSVKGRFTI SRDNSKNTLY LQMNSLRAED TAVYYCARDD YGGKGGAFDI WGQGTMVTVS  120
SASTKGPSVF PLAPSSKS                                                138

M6-D03                  LC
QDIQMTQSPS SLSASVGDRV TITCRASQSI SSYLNWYQQK PGKAPKLLIY AASSLQSGVP   60
SRFSGSGSGT DFTLTISSLQ PEDFATYYCQ QSYSTLWTFG QGTKVEIK               108

M6-D03                  HC
EVQLLESGGG LVQPGGSLRL SCAASGFTFS KYFMGWVRQA PGKGLEWVSV IGSSGGWTSY   60
ADSVKGRFTI SRDNSKNTLY LQMNSLRAED TAVYYCARVS TAVADNDYWG QGTLVTVSSA  120
STKGPSVFPL APSSKS                                                  136

M6-D08                  LC
QDIQMTQSPS SLSASVGDRV TITCRASQSI SSYLNWYqQK PGKAPKLLIY GASSLQSGVP   60
SRFSGSGSGT DFTLTISSLQ PEDSATYYCQ QSYTRWTFGQ GTKVEIK                107

M6-D08                  HC
EVQLLESGGG LVQPGGSLRL SCAASGFTFS RYHMVWVRQA PGKGLEWVSS ISPSGGWTNY   60
ADSVKGRFTI SRDNSKNTLY LQMNSLRAED TAMYYCAREM ATIAGQFDPW GQGTLVTVSS  120
ASTKGPSVFP LAPSSKS                                                 137
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M6-G05                    LC
QDIQMTQSPS SLSASVGDRV TITCRASQSI STYLNWYQLK PGKAPKLLIY NAFSMERGVP   60
STISGSGSGT DFTLTISSLQ PEDFATYYCQ QSYTTPTTFG QGTKVEIK               108

M6-G05                    HC
EVQLLESGGG LVQPGGSLRL SCAASGFTFS RYRMVWVRQA PGKGLEWVSS IYPSGGMTAY   60
ADSVKGRFTI SRDNSKNTLY LQMNSLRAED TAVYYCARDA VGIGDAFDIW GQGTMVTVSS  120
ASTKGPSVFP LAPSSKS                                                 137

M8-C04                    LC
QSALTQPPSV SVSPGQTASI TCSGDKLGDK YTSWHQQKPG QSPVLVIYQD SKRPSGIPER   60
FSGSNSGNTA TLTISGTQAM DEADYYCQAW DSSTVFGGGT RLTVL                  105

M8-C04                    HC
EVQLLESGGG LVQPGGSLRL SCAASGFTFS YYPMQWVRQA PGKGLEWVSY IYPSGGLTSY   60
ADSVKGRFTI SRDNSKNTLY LQMNSLRAED TAVYYCARLF YGSGSVGFEY WGQGTLVTVS  120
SASTKGPSVF PLAPSSKS                                                138

M8-D05                    LC
QDIQMTQSPS FVSASVGDRV TITCRASQDI SSWLVWYQQK PGKGPKLLIY DASNLQSGVP   60
SRFSGGGSGT HFTLTISSLQ PEDFATYYCQ QADGFPLTFG GGTKVEMK               108

M8-D05                    HC
EVQLLESGGG LVQPGGSLRL SCAASGFTFS LYNMNWVRQA PGKGLEWVSS ISPSGGFTDY   60
ADSVKGRFTI SRDNSKNTLY LQMNSLRAED TAVYYCARDL DLGILDYWGQ GTLVTVSSAS  120
TKGPSVFPLA PSSKS                                                   135

M8-E06                    LC
QDIQMTQSPS SLSASVGDRV TITCRASQSI SSYLNWYQQK PGKAPKLLIY AASSLQSGVP   60
SRFSGSGSGT DFTLTISSLQ PEDFATYYCQ QSYSTLMYTF GQGTKLEIK              109

M8-E06                    HC
EVQLLESGGG LVQPGGSLRL SCAASGFTFS HYFMTWVRQA PGKGLEWVSS IVPSGGMTQY   60
ADSVKGRFTI SRDNSKNTLY LQMNSLRAED TAVYYCARDS YSSSWFDIWG QGTMVTVSSA  120
STKGPSVFPL APSSKS                                                  136

M8-G09                    LC
QDIQMTQSPS SLSASVGDTV TITCRASQGV SYYLAWFQQK PGKAPKSLIY GASSLQSGVP   60
SKFSGSGSGT VFTLTISSLQ PDDFATYYCQ QYNTYPPTFG QGTRLDIK               108

M8-G09                    HC
EVQLLESGGG LVQPGGSLRL SCAASGFTFS LYEMLWVRQA PGKGLEWVSV IYPSGGYTDY   60
ADSVKGRFTI SRDNSKNTLY LQMNSLRAED MAVYYCARSF SGFGEIDYWG QGTLVTVSSA  120
STKGPSVFPL APSSKS                                                  136

M8-H04                    LC
QDIQMTQSPS SLSASIGDRV TITCRASQYI STYLNWYEQK PGKAPKLLIY GTSSLQSGVP   60
SRFSGSGSGT EFSLTISSLQ PEDFATYYCQ QSFTTPFTFG QGTKLEIK               108

M8-H04                    HC
EVQLLESGGG LVQPGGSLRL SCAASGFTFS GYWMGWVRQA PGKGLEWVSS ISSSGGWTQY   60
ADSVKGRFTI SRDNSKNTLY LQMNSLRAED TATYYCARDD EIAAGGAFDI WGQGAMVTVS  120
SASTKGPSVF PLAPSSKS                                                138

M9-A03                    LC
QDIQMTQSPS SLSASLGDRV TITCRASQNI DIYLNWYQQT PGKAPKLLIY GAYNLQSGVP   60
SRFSGSGSGT DFTLTISSLQ PEDFGTYYCQ QSYGTPVFGQ GTKLEIK                107

M9-A03                    HC
EVQLLESGGG LVQPGGSLRL SCAASGFTFS GYFMMWVRQA PGKGLEWVSS IYSSGGYTDY   60
ADSVKGRFTI SRDNSKNTLY LQMNSLRAED TAVYYCAREV AGTYAFDIWG QGTMVTVSSA  120
STKGPSVFPL APSSKS                                                  136

M9-A08                    LC
QDIQMTQSPS SLSASVGDRV TVTCRASQRI STYLNWYQQK PGKAPKLLIS GASSLQSGVP   60
SRFSGSGSGT DFTLTISSLQ PDDFATYYCQ QSYNTPRTFG QGTKVEIR               108

M9-A08                    HC
EVQLLESGGG LVQPGGSLRL SCAASGFTFS AYEMWWVRQA PGKGLEWVSY IGSSGGSTSY   60
ADSVKGRFTI SRDNSKNTLY LQMNSLRAED TAVYYCTGGN SSSFDAFDIW GQGTMVTVSS  120
ASTKGPSVFP LAPSSKS                                                 137

M9-C08                    LC
QDIQMTQSPS SLSASVGDRV TITCRASQSI SIYVNWYQQK PGKAPNLLIF AASSLQRGVP    6
SRFSGSGSGA DFTLTISSLQ PEDFATYYCQ QSFSTPLTFG GGTKVEIK               108

M9-C08                    HC
EVQLLESGGG LVQPGGSLRL SCAASGFTFS HYGMVWVRQA PGKGLEWVSY IVPSGGLTYY   60
ADSVKGRFTI SRDNSKNTLY LQMNSLRAED TAVYYCARVD YTGDGLGYWG QGTLVTVSSA  120
STKGPSVFPL APSSKS                                                  136
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M9-C10                 LC
QDIQMTQSPS SLSASVGDRV TITCRASQGI SSYLNWYQQK PGNAPNLLIY GASSLQSGVP   60
SRFSGSGSGT DFTLTISSLQ PEDFATYYCQ ESYSTLFTFG PGTTVEIK               108

M9-C10                 HC
EVQLLESGGG LVQPGGSLRL SCAASGFTFS LYPMQWVRQA PGKGLEWVSS IGSSGGMTFY   60
ADSVKGRFTI SRDNSKNTLY LQMNSLRAED TAVYYCTREV GAAGFAFDIW GQGTMVTVSS  120
ASTKGPSVFP LAPSSKS                                                 137

M9-D08                 LC
QDIQMTQSPS SLSASVGDRV TLTCRASRTI SFYLNWYQQK AGKAPELLIY GGSSLHSGVP   60
SRFSGSGSGT DFSLTISNLQ PEDIAVYYCQ QSFSSPWTFG QGTKVEIK               108

M9-D08                 HC
EVQLLESGGG LVQPGGSLRL SCAASGFTFS WYKMMWVRQA PGKGLEWVSS IYPSGGWTNY   60
ADSVKGRFTI SRDNSKNTLY LQMNSLRAED TAVYYCTRGS PWGDDAFDIW GQGTMVTVSS  120
ASTKGPSVFP LAPSSKS                                                 137

M9-E04                 LC
QDIQMIQSPS SLSASVGDRV TITCRASQSI SGYLNWYQQR SGKAPKLLIF AASNLQTGVP   60
SRFSGSGSGT DFTLTINNLQ PEDFATYYCQ QSHTPPKTFG PGTKVDIK               108

M9-E04                 HC
EVQLLESGGG LVQPGGSLRL SCAASGFTFS EYDMMWVRQA PGKGLEWVSS IGSSGGMTYY   60
ADSVKGRFTI SRDNSKNTLY LQMNSLRAED TAVYYCARDQ VAAAAIDYWG QGTLVTVSSA  120
STKGPSVFPL APSSKS                                                  136

M9-F08                 LC
QDIQMTqSPS SLSASVGDRV TITCRASQSI SSYLNWYQQK PGKAPKLLIY AASSLQSGVP   60
SRFSGSGSGT DFTLTISSLQ PEDFATYYCQ QSYSTPPYTF GQGTKLEIK              109

M9-F08                 HC
EVQLLESGGG LVQPGGSLRL SCAASGFTFS PYAMTWVRQA PGKGLEWVSV IYPSGGFTDY   60
ADSVKGRFTI SRDNSKNTLY LQMNSLRAED TAMYYCARAS GSYLDAFDIW GQGTMVTVSS  120
ASTKGPSVFP LAPSSKS                                                 137

M9-F09                 LC
QDIQMTQSPS SLSASVGDRV TITCRASQSI SSYLNWYQQK PGKAPKLLIY AASSLQSGVP   60
SKFSGSGSGT DYTLTISSLQ PEDFATYYCQ QTYTTPWTFG QGTKVEIK               108

M9-F09                 HC
EVQLLESGGG LVQPGGSLRL SCAASGFTFS SYPMGWVRQA PGKGLEWVSR ISSSGGMTIY   60
ADSVKGRFTI SRDNSKNTLY LQMNSLRAED TAVYYCARDD WNVGMDVWGQ GTTVTVSSAS  120
TKGPSVFPLA PSSKS                                                   135

M9-F10                 LC
QDIQMTQSPS SLSASVGDRV TITCRASQSI NTYLNWYQQK PGKAPKVLIH AASTLESGVP   60
SRFSGSGSGT DFTLTISSLQ PEDFATYYCQ QSYSTPYTFG QGTKLEVR               108

M9-F10                 HC
EVQLLESGGG LVQPGGSLRL SCAASGFTFS DYDMEWVRQA PGKGLEWVSS ISPSGGSTIY   60
ADSVKGRFTI SRDNSKNTLY LQMNSLRAED TAVYYCARQG LLTAFDIWGQ GTMVTVSSAS  120
TKGPSVFPLA PSSKS                                                   135

M9-G08                 LC
QDIQMTQSPS SLSASVGDRV TITCRASQSI SSYLNWYQQK PGKAPKLLIY AASSLQSGVP   60
SRFSGSGSGT DFTLTISSLQ PEDFATYYCQ QSYSTPITFG GGTKVEIK               108

M9-G08                 HC
EVQLLESGGG LVQPGGSLRL SCAASGFTFS YYTMLWVRQA PGKGLEWVSS IYPSGGFTMY   60
ADSVKGRFTI SRDNSKNTLY LQMNSLRAED TAVYYCARVD TAMAMIDYWG QGTLVTVSSA  120
STKGPSVFPL APSSKS                                                  136

M9-H02                 LC
QDIQMTQSPS SLSASVGDRV IITCRASRSI ATYLNWYQQK PGKAPNLLIF GASTLQSGVP   60
SRFSGSGSGT DFTLTISDLQ PEDFATYYCQ QSFSDPYTFG QGTNLEMK               108

M9-H02                 HC
EVQLLESGGG LVQPGGSLRL SCAASGFTFS AYMMIWVRQA PGKGLEWVSV IYPSGGVTMY   60
ADSVKGRFTI SRDNSKNTLY LQMNSLRAED TAVYYCARGT VGASDAFDIW GQGTMVTVSS  120
ASTKGPSVFP LAPSSKS                                                 137

M9-H03                 LC
QYELTQAPSV SVAPGQTASI TCSGDKLGNR YTSWYQQKPG QSPVLVIFQD NKRPSGIPER   60
FSGSNSGNTA TLTISGTQAM DEADYYCQAL DSNTYVFGTG TKVTVL                 106

M9-H03                 HC
EVQLLESGGG LVQPGGSLRL SCAASGFTFS WYSMGWVRQA PGKGLEWVSY IVPSGGYTMY   60
ADSVKGRFTI SRDNSKNTLY LQMNSLRAED TAVYYCARDP GVSYYYYGMD VWGQGTTVTV  120
SSASTKGPSV FPLAPSSKS                                               139
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M9-H04                  LC
QDIQMTQSPS SLSASVGDRV TITCRASQSI SSYLNWYQQK PGKAPKLLIY AASSLQSGVP   60
SRFSGSGSGT DFTLTISSLQ PEDFATYYCQ QSYSTPPTFG QGTRLEIK               108

M9-H04                  HC
EVQLLESGGG LVQPGGSLRL SCAASGFTFS AYTMWWVRQA PGKGLEWVSS IWPSGGSTFY   60
ADSVKGRFTI SRDNSKNTLY LQMNSLRAED TAVYYCARTY DSSAGEVDYW GQGTLVTVSS  120
ASTKGPSVFP LAPSSKS                                                 137

M10-A03                 LC
QDIQMTQSPS SLSASVGDRV TITCRASQRI SFYLNWFQQK PGKAPNLLIY GASSLQSGVP   60
SRFSGSGSGT DFTLTISSLQ PKDFGTYYCQ QTFSTPNTFG QGTKLEIK               108

M10-A03                 HC
EVQLLESGGG LVQPGGSLRL SCAASGFTFS PYPMQWVRQA PGKGLEWVSS ISSSGGMTEY   60
ADSVKGRFTI SRDNSKNTLY LQMNSLRAED TAVYYCARDD YGGKGGAFDI WGQGTMVTVS  120
SASTKGPSVF PLAPSSKS                                                138

M10-A12                 LC
QDIQMTQSPL SLSAFVGDRV TITCRASRDI SVYLNWYQLK SGKAPKLLIY GASSLQSGVP   60
SRFSGSGSGT DFTLTITSLQ PEDFATYYCQ QSYSIPFTFG GGTKVETK               108

M10-A12                 HC
EVQLLESGGG LVQPGGSLRL SCAASGFTFS LYLMHWVRQA PGKGLEWVSS IYSSGGFTTY   60
ADSVKGRFTI SRDNSKNTLY LQMNSLRAED TAVYYCARDT DYGMDVWGQG TTVTVSSAST  120
KGPSVFPLAP SSKS                                                    134

M10-B09                 LC
QDIQMTQSPS SLSASVGDGV TITCRASQSI STYLNWYQQR PGKAPKLLIY GASSLQSGVP   60
SRFSGSGSGT DFTLTISSLQ REDFATYYCQ QSFSTPWTFG QGTRVEIK               108

M10-B09                 HC
EVQLLESGGG LVQPGGSLRL SCAASGFTFS WYEMSWVRQA PGKGLEWVSR IWPSGGVTMY   60
ADSVKGRFTI SRDNSKNTLY LqMNSLRAED TAVYYCTRTS ITTVGMDVWG QGTTVTVSSA  120
STKGPSVFPL APSSKS                                                  136

M10-C11                 LC
QDIQMTQSPS SLSASVGDRV TITCRASQSI SIYLNWYQQK PEKAPKLLIF AASTLQSGVP   60
SRFSGSGSGT DFTLTISNLQ PEDFATYYCQ QSHSIPPTFG LGTKVEVK               108

M10-C11                 HC
EVQLLESGGG LVQPGGSLRL SCAASGFTFS MYPMMWVRQA PGKGLEWVSY ISPSGGMTDY   60
ADSVKGRFTI SRDNSKNTLY LQMNSLRAED MAVYYCARVA GSSDAFDIWG QGTMVTVSSA  120
STKGPSVFPL APSSKS                                                  136

M10-D11                 LC
QDIQMTQSPL SLPVTPGEPA SISCRSSQSL LHSNGYNYLD WYLQKPGQSP QLLIYLGSNR   60
ASGVPDRFSG SGSGTDFTLK ISRVEAEDVG VYYCMQALQT PLTFGPGTKV HIK          113

M10-D11                 HC
EVQLLESGGG LVQPGGSLRL SCAASGFTFS AYPMNWVRQA PGKGLEWVSR ISSSGGNTSY   60
ADSVKGRFTI SRDNSKNTLY LQMNSLRAED TAVYYCALGY LGYWGQGTLV TVSSASTKGP  120
SVFPLAPSSK S                                                       131

M10-E06                 LC
QDIQMTQSPS SLSASVGDRV TITCRASQSI STYLNWYQQK PGKAPKLLIY GASSLQSGVP   60
SRFSGSGSGT DFTLTISSLQ PEDFTIYYCQ QSYSDPYTFG QGTKLDIK               108

M10-E06                 HC
EVQLLESGGG LVQPGGSLRL SCAASGFTFS LYRMFWVRQA PGKGLEWVSS IWSSGGPTMY   60
ADSVKGRFTI SRDNSKNTLY LQMNSLRAED TAVYYCAREY PSTYYFDYWG QGTLVTVSSA  120
STKGPSVFPL APSSKS                                                  136

M10-F09                 LC
QDIQMTQSPS SLSASVGDRV TITCRASQTI DDDLIWYQQK PGRAPKLLIY AASSLQSGVP   60
SRFSGSGSGT DFTLTITSLQ PEDFATYYCQ QSYNIPRTFG QGTKLESK               108

M10-F09                 HC
EVQLLESGGG LVQPGGSLRL SCAASGFTFS NYDMMWVRQA PGKGLEWVSY ISPSGGFTRY   60
ADSVKGRFTI SRDNSKNTLY LQMNSLRAED TATYYCAKDI YYYNWGPSHY FDSWGQGTLV  120
TVSSASTKGP SVFPLAPSSK S                                            141

M10-G09                 LC
QDIQMTQSPS SLSASVGDSV TITCRASQSI SGYINWYQQK AGKAPKLLIY AASSLQSGVP   60
SRFSGSGSGT HFTLTISSLQ PEDFATYYCQ QYVSYPFTFG PGTKVDIK               108

M10-G09                 HC
EVQLLESGGG LVQPGGSLRL SCAASGFTFS QYGMQWVRQA PGKGLEWVSS IRSSGGATRY   60
ADSVKGRFTI SRDNSKNTLY LQMNSLRAED TAVYYCARDG YYDSSGYPDY WGQGTLVTVS  120
SASTKGPSVF PLAPSSKS                                                138
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M11-A10               LC
QDIQMTQSPS SLSASVGDRV AITCRASQSI DTYLNWYQQK PGKAPKLLIY DASNLEIGVP   60
SRFSGSGSGT DFTFIINSLQ PEDVATYYCQ HYLYAPYSFG QGTKLEIK               108

M11-A10               HC
EVQLLESGGG LVQPGGSLRL SCAASGFTFS NYWMMWVRQA PGKGLEWVSG IGSSGGFTSY   60
ADSVKGRFTI SRDNSKNTLY LQMNSLRAED TAVYYCAKGS YSDYGVFESW GQGTLVTVSS  120
ASTKGPSVFP LAPSSKS                                                 137

M11-E01               LC
QDIQMTQSPS SLSASVGDRV TITCRASQSI SSYLNWYQQK PGKAPKLLIY AASSLQSGVP   60
SRFSGSGSGT DFTLTISSLQ PEDFATYYCQ QSYSTPPTFG QGTKVEIK               108

M11-E01               HC
EVQLLESGGG LVQPGGSLRL SCAASGFTFS TYEMYWVRQA PGKGLEWVSG IGSSGGMTMY   60
ADSVKGRFTI SRDNSKNTLY LQMNSLRAED TAMYYCAREQ PGIAALQFWG QGTLVTVSSA  120
STKGPSVFPL APSSKS                                                  136

M11-E04               LC
QDIQMTQSPS SLSASVGDRV TITCRASQSI SIYLTWYQHR PGKAPNLLIY GAATLQTGVP   60
SRFSGSGSGT DFTLTIRGLQ PEDFATYYCQ QTFSLPRTFG QGTKLEIK               108

M11-E04               HC
EVQLLESGGG LVQPGGSLRL SCAASGFTFS MYHMNWVRQA PGKGLEWVSG IVSSGGVTFY   60
ADSVKGRFTI SRDNSKNTLY LQMNSLRAED TAVYYCARIT TVTTGGAFDI WGQGTMVTVS  120
SASTKGPSVF PLAPSSKS                                                138

M11-E05               LC
QDIQMTQSPS SLSASVGDTV TITCRTSQTI NNYLNWYQQR PGEAPKVLIY ATHTLESGVP   60
SRFSGSGSGT DFTLTIGSLQ PEDFATYYCQ QSFAFPYTFG QGTKVEIT               108

M11-E05               HC
EVQLLESGGG LVQPGGSLRL SCAASGFTFS WYTMGWVRQA PGKGLEWVSW IYFGGLTTYA   60
DSVKGRFTIS RDNSKNTLYL QMNSLRAEDT AVYYCARLGG PLDAFDIWGQ GTMVTVSSAS  120
TKGPSVFPLA PSSKS                                                   135

M11-E06               LC
QDIQMTQSPS SLSASIGDRV TISCRASRGI GTYLNWYQQH AGKAPKLLIR AASSLETGVP   60
PRFSGSGSGT DFTLTISSLQ SDDFATYYCQ ESFTNVYNFG QGTKLEIK               108

M11-E06               HC
EVQLLESGGG LVQPGGSLRL SCAASGFTFS QYAMHWVRQA PGKGLEWVSS IYPSGGFTLY   60
ADSVKGRFTI SRDNSKNTLY LQMNSLRAED TAMYYCARGG WLAGGELLNW GQGTLVTVSS  120
ASTKGPSVFP LAPSSKS                                                 137

M11-G09               LC
QDIQMTQSPS SLSASVGDRV TITCRTSQGI NHYLNWYQQK PGKAPKILVF AASELQTGVP   60
SRFSGTGSGT SYTLTITSLQ PEDVATYYCQ QTYTSPYTFG QGTKLEVK               108

M11-G09               HC
EVQLLESGGG LVQPGGSLRL SCAASGFTFS LYNMTWVRQA PGKGLEWVSY IYPSGGGTHY   60
ADSVKGRFTI SRDNSKNTLY LQMNSLRAED TAVYYCARDT GFWSADAFDI WGQGTMVTVS  120
SASTKGPSVF PLAPSSKS                                                138

M11-G12               LC
QDIQMTQSPS SLSAFVGDRV SITCRASQTI SVYVNWYQHK SGQAPKLLIY GASSLQSGVP   60
SRFSGSGSGT DFTLTISSLQ PEDFATYFCQ QSYSIPFTFG GGTDVQIR               108

M11-G12               HC
EVQLLESGGG LVQPGGSLRL SCAASGFTFS QYPMNWVRQA PGKGLEWVSS ISSSGGFTTY   60
ADSVKGRFTI SRDNSKNTLY LQMNSLRAED TAVYYCAREE QQGGFDYWGQ GTLVTVSSAS  120
TKGPSVFPLA PSSKS                                                   135

M12-A08               LC
QDIQMTQSPS SLSASVGDRV TITCRASQSI SRYLNWYQQK PGKAPKLLIY AASTLETGVP   60
SRFSGSGSGT DFTLTITTLQ PEDFVIYYCQ QSYSTPYTFG QGTKLEIK               108

M12-A08               HC
EVQLLESGGG LVQPGGSLRL SCAASGFTFS WYYMGWVRQA PGKGLEWVSW IVSSGGLTLY   60
ADSVKGRFTI SRDNSKNTLY LQMNSLRAED TAMYYCARTT VTTGDAFDIW GQGTMVTVSS  120
ASTKGPSVFP LAPSSKS                                                 137

M12-B04               LC
QDIQMTQSPS SLSASVGDRV TITCRASQGI RNDLGWYQHK PGKAPKLLIY AASILQSGVP   60
SRFSGTASGT DFTLTISSLQ PEDFATYFCL QDYEYPLTFG GGTKLDIK               108

M12-B04               HC
EVQLLESGGG LVQPGGSLRL SCAASGFTFS LYSMYWVRQA PGKGLEWVSR IRPSGGGTVY   60
ADSVKGRFTI SRDNSKNTLY LQMNSLRAED TAVYYCARDP LYSSGDVWGQ GTTVTVSSAS  120
TKGPSVFPLA PSSKS                                                   135
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M12-009               LC
QDIQMTQSPS SLSASVGDRV TITCRASQSI GIYLNWYHQK PGKAPNLLIY GASSLQSGVP   60
SRFSGSGSGT DFTLTISSLQ PGDFATYYCQ HSYSTPFTFG GGTKVEIK               108

M12-009               HC
EVQLLESGGG LVQPGGSLRL SCAASGFTFS SYAMVWVRQA PGKGLEWVSS IGSSGGFTLY   60
ADSVKGRFTI SRDNSKNTLY LQMNSLRAED TAVYYCASMN LGGGDAFDIW GQGTMVTVSS  120
ASTKGPSVFP LAPSSKS                                                 137

M12-C10               LC
QSALTQPPSV SVSPGQTASI TCSGDKLGEK YVSWYQQKPG QSPVVVIYQD NKRPSGIPER   60
FSGSNSGNTA TLTISGTQAV DEADYYCQAW DSYTVVFGGG SKLTVLGQPK             110

M12-C10               HC
EVQLLESGGG LVQPGGSLRL SCAASGFTFS DYEMHWVRQA PGKGLEWVSG ISPSGGKTQY   60
ADSVKGRFTI SRDNSKNTLY LQMNSLRAED TAVYYCARDL KWGGRGSPDW YFDLWGRGTL  120
VTVSSASTKG PSVFPLAPSS KS                                           142

M12-D10               LC
QDIQMTQSPS SLSASVGDRV TITCRASQSI SSYLNWYQQK PGKAPKLLIY AASSLQSGVP   60
SRFSGSGSGT DFTLTISSLQ PEDFATYYCQ QSYSTPPTFG GGTKVEIK               108

M12-D10               HC
EVQLLESGGG LVQPGGSLRL SCAASGFTFS NYPMDWVRQA PGKGLEWVSS ISSSGGWTNY   60
ADSVKGRFTI SRDNSKNTLY LQMNSLRAED TAVYYCATDT SGSYLGFDYW GQGTLVTVSS  120
ASTKGPSVFP LAPSSKS                                                 137

M12-E06               LC
QDIQMTQSPS SLSASVGDRV SITCRASQSI STYLNWYQHK PGKAPTLLIY GAFSLQSGVP   60
SRFSGSGSGT DFALTISSLQ PEDFATYYCQ QSHSTPPTFG QGTRVEIK               108

M12-E06               HC
EVQLLESGGG LVQPGGSLRL SCAASGFTFS QYKMLWVRQA PGKGLEWVSG IGPSGGLTAY   60
ADSVKGRFTI SRDNSKNTLY LQMNSLRAED TAVYYCARAP WFGELGMDVW GQGTTVTVSS  120
ASTKGPSVFP LAPSSKS                                                 137

M27-A10               LC
QDIQMTQSPS SLSASVGDRV TITCRASQSI SAYLNWYQQK PGKAPQLLMY GVGSLQSGVP   60
SRFSGSGSGT DFTLTISSLQ PEDFATYFCQ QGYTTPVTFG GGTKVEIK               108

M27-A10               HC
EVQLLESGGG LVQPGGSLRL SCAASGFTFS WYRMDWVRQA PGKGLEWVSS IWPSGGLTSY   60
ADSVKGRFTI SRDNSKNTLY LQMNSLRAED TAVYYCARGW APGGDAFDIW GQGTMVTVSS  120
ASTKGPSVFP LAPSSKS                                                 137

M27-B01               LC
QDIQMTQSPS SLSASVGDRV TITCRASQSI SSYLNWYQQK PGKAPKLLIY AASSLQSGVP   60
SRFSGSGSGT DFTLTISSLQ PEDFATYYCQ QSYSTPYTFG QGTKLEIK               108

M27-B01               HC
EVQLLESGGG LVQPGGSLRL SCAASGFTFS DYTMWWVRQA PGKGLEWVSS ISSSGGITFY   60
ADSVKGRFTI SRDNSKNTLY LQMNSLRAED TAVYYCARSA DTAMGGAFDI WGQGTMVTVS  120
SASTKGPSVF PLAPSSKS                                                138

M27-B12               LC
QYELTQPPAV SVSPGQTATI TCSGDKLGDE YAAWYQQKPG QSPVLVIYQD RKRPSGIPER   60
FSGSNFGNTA TLTITGTQVM DEADYYCQAW GKRNVVFGGG TKLTVL                 106

M27-B12               HC
EVQLLESGGG LVQPGGSLRL SCAASGFTFS WYQMMWVRQA PGKGLEWVSS ISPSGGITEY   60
ADSVKGRFTI SRDNSKNTLY LQMNSLRAED TAVYYCARDR SSGWYYYGMD VWGQGTTVTV  120
SSASTKGPSV FPLAPSSKS                                               139

M27-E03               LC
QDIQMTQSPS SLSASVGDRV TITCRASQSI SSYLNWYQQK PGKAPKLLIY AASSLQSGVP   60
SRFSGSGSGT DFTLTISSLQ PEDFATYYCQ QSYSTPRTFG QGTKVEIK               108

M27-E03               HC
EVQLLESGGG LVQPGGSLRL SCAASGFTFS SYMMHWVRQA PGKGLEWVSG IYPSGGWTDY   60
ADSVKGRFTI SRDNSKNTLY LQMNSLRAED TATYYCARLV AGLDAFDIWG QGTMVTVSSA  120
STKGPSVFPL APSSKS                                                  136

M27-F04               LC
QDIQMTQSPS SLSASVGDRV TITCRASQSI SSYLNWYQQK PGKAPKLLIY AASSLQSGVP   60
SRFSGSGSGT DFTLTISSLQ PEDFATYYCQ QSYSTPPTFG QGTKVEIK               108

M27-F04               HC
EVQLLESGGG LVQPGGSLRL SCAASGFTFS WYPMTWVRQA PGKGLEWVSS IGPSGGQTIY   60
ADSVKGRFTI SRDNSKNTLY LQMNSLRAED TAVYYCTTEY GDYGGGFDPW GQGTLVTVSS  120
ASTKGPSVFP LAPSSKS                                                 137
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M27-F11              LC
QDIQMTQSPS FLSASVGDRV TITCRASQGI SSYLAWYQQK PGKAPKLLIY AASSLQSGVP   60
SRFSGSGSGT DFTLTISSLQ PEDFATYYCQ QSYNTLRTFG PGTKVDLK               108

M27-F11              HC
EVQLLESGGG LVQPGGSLRL SCAASGFTFS SYHMMWVRQA PGKGLEWVSS IYPSGGATMY   60
ADSVKGRFTI SRDNSKNTLY LQMNSLRAED TAMYYCARDG YHYGDYTYFQ HWGQGTLVTV  120
SSASTKGPSV FPLAPSSKS                                               139

M27-G01              LC
QDIQMTQSPS SLSASVGDRV TITCRASQSI STYLNWYQQK PGKAPKLLIY GASSLQSGVP   60
SRFSGSGSGT DFTLTISSLQ PEDFTIYYCQ QSYSDPYTFG QGTKLDIK               108

M27-G01              HC
EVQLLESGGG LVQPGGSLRL SCAASGFTFS LYRMFWVRQA PGKGLEWVSS IWSSGGPTMY   60
ADSVKGRFTI SRDNSKNTLY LQMNSLRAED TAVYYCAREY PSTYYFDYWG QGTLVTVSSA  120
STKGPSVFPL APSSKS                                                  136

M27-G04              LC
QDIQMTQSPS SLSASVGDRV TITCRASQRI SYYLTWYQQK PGKVPKLLIY AASSLESGVP   60
SRFSGSGSGT DFTLTISNLQ PEDFATYYCQ QAFSTPFTFG GGTKVEIK               108

M27-G04              HC
EVQLLESGGG LVQPGGSLRL SCAASGFTFS AYYMVWVRQA PGKGLEWVSY ISPSGGQTQY   60
ADSVKGRFTI SRDNSKNTLY LQMNSLRAED TAVYYCAREA ISSSSFDYWG QGTLVTVSSA  120
STKGPSVFPL APSSKS                                                  136

M27-G09              LC
QDIQMTQSPS SVSASVGDRI TITCRTRQSI SNYLNWYQQK PGEPPKLLIF AASSLQSGVP   60
SRFSGSGTGT EFTLTISSLQ PEDLAIYYCQ QSYDIPFTFG QGTKLEIK               108

M27-G09              HC
EVQLLESGGG LVQPGGSLRL SCAASGFTFS EYDMAWVRQA PGKGLEWVSY IVSSGGFTSY   60
ADSVKGRFTI SRDNSKNTLY LQMNSLRAED TAVYYCTTWA GWIAAADYWG QGTLVTVSSA  120
STKGPSVFPL APSSKS                                                  136

M27-H10              LC
QDIQMTQSPS SLSASVGDRV TITCRASQSI SNYLNWYQQK PGKAPKFLIY AASSLQSGVP   60
SRFSGSGSGT DFTLSISSLQ PEDFATYYCQ QSYSTPQTFG QGTKVEMK               108

M27-H10              HC
EVQLLESGGG LVQPGGSLRL SCAASGFTFS AYQMAWVRQA PGKGLEWVSV IYSSGGYTDY   60
ADSVKGRFTI SRDNSKNTLY LQMNSLRAED TAVYYCARHN WNDGAFDIWG QGTMVTVSSA  120
STKGPSVFPL APSSKS                                                  136

M28-A01              LC
QDIQMTQSPS SLSASVGDRV TITCRASQSI SSYLNWYQQK PGKAPKLLIY AASSLQSGVP   60
SRFSGSGSGT DFTLTISSLQ PEDFATYYCQ QSYSTLTFGG GTKVEIK                107

M28-A01              HC
EVQLLESGGG LVQPGGSLRL SCAASGFTFS WYAMHWVRQA PGKGLEWVSG IYSSGGYTKY   60
ADSVKGRFTI SRDNSKNTLY LQMNSLRAED TAVYYCARDL SNGDDVFDIW GQGTMVTVSS  120
ASTKGPSVFP LAPSSKS                                                 137

M28-C03              LC
QDIQMTQSPS SLSASVGDRV TITCRASQSI NFYLNWYQQK PGKAPKLLIY VASSLESGVP   60
SRFSGSASGT EFTLTISSLQ PEDFATYYCL QSYSAPYTFG QGTKVEIT               108

M28-C03              HC
EVQLLESGGG LVQPGGSLRL SCAASGFTFS YYQMGWVRQA PGKGLEWVSS IYPSGGMTDY   60
ADSVKGRFTI SRDNSKNTLY LQMNSLRAED TAVYYCTRGS PWGDDAFDIW GQGTMVTVSS  120
ASTKGPSVFP LAPSSKS                                                 137

M28-D02              LC
QDIqMTQSPS SLSASEGDMV TITCRTSRRI GTYLNWYQQK PGKAPKLLIY GASSLQSGVP   60
SRFSGSGSGT DFTLTVSSLQ PEDVGTYYCQ QSFSSPWTFG PGTKVEIK               108

M28-D02              HC
EVQLLESGGG LVQPGGSLRL SCAASGFTFS WYPMQWVRQA PGKGLEWVSY IYPSGGGTDY   60
ADSVKGRFTI SRDNSKNTLY LQMNSLRAED TATYYCATSS GWLGDAFDIW GQGTMVTVSS  120
ASTKGPSVFP LAPSSKS                                                 137

M28-D12              LC
QDIQMTQSPS SLSASVGDRV TITCRASQSI ATYLNWYQQK PGRAPKLLIY AASSLQSGVP   60
SRFVGGGSGS GTHFTLTISS LQPEDFATYY CQQSYSTRET FGQGTKVEIK             110

M28-D12              HC
EVQLLESGGG LVQPGGSLRL SCAASGFTFS WYTMHWVRQA PGKGLEWVSV IYPSGGPTSY   60
ADSVKGRFTI SRDNSKNTLY LQMNSLRAED TATYYCARDG SGSYLGFDYW GQGTLVTVSS  120
ASTKGPSVFP LAPSSKS                                                 137
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M28-E01              LC
QDIQMTQSPS SLSASVGDRV TITCRASQSI SSYLNWYQQK PGKAPKLLIY AASSLQSGVP   60
SKFSGSGSGT DYTLTISSLQ PEDFATYYCQ QTYTTPWTFG QGTKVEIK               108

M28-E01              HC
EVQLLESGGG LVQPGGSLRL SCAASGFTFS SYPMGWVRQA PGKGLEWVSR ISSSGGMTIY   60
ADSVKGRFTI SRDNSKNTLY LQMNSLRAED TAVYYCARDD WNVGMDVWGQ GTTVTVSSAS  120
TKGPSVFPLA PSSKS                                                  135

M28-E11              LC
QDIQMTQSPS SVSASVGDRV TINCRASQDI SNWLAWYQQK PGKAPNLLIY AASSLQTGAP   60
SRFSGSGSGT DFTLTISSLQ PEDFGTYVCQ QSYSLPWTFG LGTKVEVR               108

M28-E11              HC
EVQLLESGGG LVQPGGSLRL SCAASGFTFS LYDMTWVRQA PGKGLEWVSG ISSSGGVTIY   60
ADSVKGRFTI SRDNSKNTLY LQMNSLRAED TAVYYCARTY YYDSSGYADA FDIWGQGTMV  120
TVSSASTKGP SVFPLAPSSK S                                           141

M28-F01              LC
QDIQMTQSPS SLSASVGDRV TITCRASQSI NTYLNWYQQK PGKAPKVLIH AASTLESGVP   60
SRFSGSGSGT DFTLTISSLQ PEDFATYYCQ QSYSTPPTFG QGTKVEIK               108

M28-F01              HC
EVQLLESGGG LVQPGGSLRL SCAASGFTFS VYLMHWVRQA PGKGLEWVSG ISPSGGYTQY   60
ADSVKGRFTI SRDNSKNTLY LQMNSLRAED TAVYYCARPG GLDAFDIWGQ GTMVTVSSAS  120
TKGPSVFPLA PSSKS                                                  135

M28-F05              LC
QDIQMTQSPS SLSASVGDRV TITCRASQSI SSYLNWYQQK PGKAPKLLIY AASSLQSGVP   60
SRFSGSGSGT DFTLTISSLQ PEDFATYYCQ QSYSTPLTFG GGTKVEIK               108

M28-F05              HC
EVQLLESGGG LVQPGGSLRL SCAASGFTFS RYIMWWVRQA PGKGLEWVSG IYSSGGYTQY   60
ADSVKGRFTI SRDNSKNTLY LQMNSLRAED TAMYYCAREL EGLGGFDYWG QGTLVTVSSA  120
STKGPSVFPL APSSKS                                                 136

M28-F07              LC
QDIQMTQSPS SVSASVGDRV TITCRASQGI SSWLAWYQQK PGKAPKLLIY ATSGLQSGVP   60
SRFSGSGSGT DFTLTISSLQ PEDFATYYCQ QAKSFPLTFG GGTRVEIK               108

M28-F07              HC
EVQLLESGGG LVQPGGSLRL SCAASGFTFS DYTMYWVRQA PGKGLEWVSS IVPSGGHTLY   60
ADSVKGRFTI SRDNSKNTLY LQMNSLRAED TAVYYCAKDH LSSWYGGFFD YWGQGTLVTV  120
SSASTKGPSV FPLAPSSKS                                              139

M29-C07              LC
QDIQMTQSPS SLSASVGDRV TITCRASQSI SSYLNWYQQK PGKAPKLLIY AASSLQSGVP   60
SRFSGSGSGT DFTLTISSLQ PEDFATYYCQ QSYSTRYTFG QGTKLEIK               108

M29-C07              HC
EVQLLESGGG LVQPGGSLRL SCAASGFTFS GYDMMWVRQA PGKGLEWVSV ISSSGGNTAY   60
ADSVKGRFTI SRDNSKNTLY LQMNSLRAED TAVYYCARES SGLYYFDYWG QGTLVTVSSA  120
STKGPSVFPL APSSKS                                                 136

M29-D10              LC
QDIQMTQSPS SLSASVGDTV SITCRASQSI TIYLNWYQHK PGKAPNLLIY GASNLHSGVP   60
SRFSGSGSGT DFTLTISSLQ PEDFATYYCQ QSYDTPLTFG GGTKVEIK               108

M29-D10              HC
EVQLLESGGG LVQPGGSLRL SCAASGFTFS WYPMYWVRQA PGKGLEWVSS IGSSGGPTPY   60
ADSVKGRFTI SRDNSKNTLY LQMNSLRAED TAVYYCARWA DYGGSLDYWG QGTLVTVSSA  120
STKGPSVFPL APSSKS                                                 136

M29-E02              LC
QSVLTQPPSV SEAPRQRVTI SCSGSSSNIG NNAVSWYQQL PGKAPKLLIY YDDLLPSGVS   60
DRFSGSKSGT SASLAISGLR SEDEADYYCA AWDDSLNGFV FGTGTKVTVL             110

M29-E02              HC
EVQLLESGGG LVQPGGSLRL SCAASGFTFS RYPMMWVRQA PGKGLEWVSV IYPSGGDTFY   60
ADSVKGRFTI SRDNSKNTLY LQMNSLRAED TAVYYCASGD DYLWEAAVYW GQGTLVTVSS  120
ASTKGPSVFP LAPSSKS                                                137

M29-G08              LC
QDIQMTQSPA TLSASPGETV TLSCRASQNI GNDVAWYRQR PGQAPRLLIH HASTRAYGIP   60
ARLRGSGSAT EFTLTITSLE PEDFAIYYCQ QFYDWPAHTF ALGTRLEIKR             110

M29-G08              HC
EVQLLESGGG LVQPGGSLRL SCAASGFTFS YYHMWWVRQA PGKGLEWVSG ISPSGGFTFY   60
ADSVKGRFTI SRDNSKNTLY LQMNSLRAED TAVYYCARDY YYDSSGYSPL GYWGQGTLVT  120
VSSASTKGPS VFPLAPSSKS                                             140
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M29-G10                LC
QDIQMTQSPS SLSSSVGDSA TITCRASQSI SIYLNWYQQK PGKAPKILIY GASQLESGVP   60
SRFSGSGSGT DFTLTVSGLQ PEDFATYWCQ QSYNVPYTFG QGTKLEIK               108

M29-G10                HC
EVQLLESGGG LVQPGGSLRL SCAASGFTFS FYKMIWVRQA PGKGLEWVSS ISSSGGSTQY   60
ADSVKGRFTI SRDNSKNTLY LQMNSLRAED TAVYYCARDR VDLGYLDYWG QGTLVTVSSA  120
STKGPSVFPL APSSKS                                                  136

M74-A07                LC
QDIQMTQSPS SLSASVRDRV TITCRTSQNI NTYLNWYYQA PGRAPKLLIF GVSSLHRGVS   60
SRFSGSGDGT EFTLTISSLQ PEDIGTYFCQ QSYSSPWTFG QGTKVEIK               108

M74-A07                HC
EVQLLESGGG LVQPGGSLRL SCAASGFTFS QYLMMWVRQA PGKGLEWVSS IYPSGGYTSY   60
ADSVKGRFTI SRDNSKNTLY LQMNSLRAED TAVYYCARVS TAVADNDYWG QGTLVTVSSA  120
STKGPSVFPL APSSKS                                                  136

M76-F02                LC
QDIQMTQSPS SLSASVGDRV TITCRASQTI DNYLHWYQQK PGKAPKVLIH DASSLQSGVP   60
PRFSGSGSGT DFTLTISSLQ PEDFATYYCQ QSYDTPQYTF GQGTKLEIK              109

M76-F02                HC
EVQLLESGGG LVQPGGSLRL SCAASGFTFS LYDMNWVRQA PGKGLEWVSG ISPSGGQTMY   60
ADSVKGRFTI SRDNSKNTLY LQMNSLRAED TAVYYCARQP MISAFDIWGQ GTMVTVSSAS  120
TKGPSVFPLA PSSKS                                                   135

M76-G02                LC
QDIQMTQSPS SLSASVGDRV TITCRASQSI SSYLNWYQQK PGKAPKLLIY AASSLQSGVP   60
SRFSGSGSGT DFTLTISSLQ PEDFATYYCQ QSYSTPPWTF GQGTKVEIK              109

M76-G02                HC
EVQLLESGGG LVQPGGSLRL SCAASGFTFS LYAMWWVRQA PGKGLEWVSY ISSSGGFTSY   60
ADSVKGRFTI SRDNSKNTLY LQMNSLRAED TAVYYCARYR VGVAATDYWG QGTLVTVSSA  120
STKGPSVFPL APSSKS                                                  136

M76-G06                LC
QDIQMTQSPS SLSASVRDRV TITCRASQSI STYLNWYQQK PGEAPKLLVF AASSLQSGVP   60
SRFSGSGSGT DFTLSISSLQ PEDFATYYCQ QSYSTPHTFG QGAKVEIK               108

M76-G06                HC
EVQLLESGGG LVQPGGSLRL SCAASGFTFS GYIMHWVRQA PGKGLEWVSW IYPSGGWTEY   60
ADSVKGRFTI SRDNSKNTLY LQMNSLRAED TAVYYCARDA PGVGAIDYWG QGTLVTVSSA  120
STKGPSVFPL APSSKS                                                  136

M76-H02                LC
QDIQMTQSPS SLSASEGDRV TITCRASQDI SVYLNWYQMK SGKAPKLLIY GGASLQSGVP   60
ARFSGSGYGT DFTLTITDLR PEDFATYYCQ QSYSLPFTFG GGTKVEIK               108

M76-H02                HC
EVQLLESGGG LVQPGGSLRL SCAASGFTFS MYWMQWVRQA PGKGLEWVSY IYPSGGPTKY   60
ADSVKGRFTI SRDNSKNTLY LQMNSLRAED TAVYYCARPS GSYGDAFDIW GQGTMVTVSS  120
ASTKGPSVFP LAPSSKS                                                 137

M77-C07                LC
QDIQMTQSPS TLSASVGDRV TITCRASQNI SSYLNWYQQK PGKAPKLLIY AASSLQSGVP   60
SRFSGSGSGT DFTLTISSLQ PEDFATYSCQ QSYSTPRTFG QGTKVEIK               108

M77-C07                HC
EVQLLESGGG LVQPGGSLRL SCAASGFTFS LYIMGWVRQA PGKGLEWVSG IYPSGGFTMY   60
ADSVKGRFTI SRDNSKNTLY LQMNSLRAED TAVYYCARES SGVAAPDYWG QGTLVTVSSA  120
STKGPSVFPL APSSKS                                                  136

M77-H04                LC
QDIQMTQSPL SLPVTPGEPA SISCRSSQSL LHSRGYNYLD WYLQKPGQSP QLLIYLGSNR   60
ASGVPDRFSG SGSGTDFTLK ISRVEAEDVG VYYCMQALQR RTFGQGTKLE IK          112

M77-H04                HC
EVQLLESGGG LVQPGGSLRL SCAASGFTFS YYTMIWVRQA PGKGLEWVSG IRSSGGGTRY   60
ADSVKGRFTI SRDNSKNTLY LQMNSLRAED TAVYYCAKDG SRYSYGSIYY YYGMDAWGQG  120
TTVTVSSAST KGPSVFPLAP SSKS                                         144
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Example 2

Lead Antibody Inhibitors

Antibodies were selected as lead plasma kallikrein inhibitors on the basis of apparent inhibition constant ($K_{i,app}$), specificity with respect to lack of inhibition of other serine proteases, inhibition of bradykinin generation, and lack of binding to plasma prekallikrein (Table 3). Plasma kallikrein circulates in the plasma as an inactive zymogen (prekallikrein) at a concentration of approximately 500 nM. Antibodies that bound prekallikrein may be rendered inaccessible towards active plasma kallikrein inhibition and could substantially increase the in vivo dose required for efficacy. Therefore, a surface plasmon resonance (SPR) assay was used to identify antibodies that do not bind prekallikrein (data not shown). Specifically, human IgGs (X81-B01, M162-A04 (R84-H05); M160-G12 (R84-D02); and M142-H08) were captured on a CM5 chip using an anti-human Fc surface and 100 nM of plasma kallikrein or 100 nM or 500 nM prekallikrein. The prekallikrein was treated with aprotinin-sepharose to remove active plasma kallikrein. The prekallikrein used for X81-B01 was buffer exchanged into the exact preparation of SPR running buffer (HEPES buffered saline) to avoid the refractive index shift that was observed with three other antibodies that were tested: M162-A04 (R84-H05); M160-G12 (R84-D02); and M142-H08.

Of the antibodies listed in Table 3, only M142-H08 inhibits human plasma kallikrein with a subnanomolar $K_{i,app}$. However, when M142-H08 was produced as an IgG it was found to be cleaved in the CDR3 of the heavy chain. Consequently, we decided to undertake two approaches to improve the affinity: 1) affinity maturation of M162-A04 and M160-G12 using a novel form of light chain shuffling called ROLIC (Rapid Optimization of Light Chains) (see, e.g., WO 2009/102927 and U.S. 2009-0215119); and 2) sequence optimization of M142-H08 in order to prevent the cleavage of the IgG that occurs while retaining the binding and inhibitor properties of M142-H08.

TABLE 3

Top Ranking Antibody Inhibitors of PKal Before Affinity Maturation or Sequence Optimization

Criteria	M162-A04	M160-G12	M142-H08[a]
$K_{i,app}$ human pKal	2 nM (as an IgG)	5.6 nM (as an IgG)	0.6 nM (as a Fab)
$K_{i,app}$ rodent pKal	2 nM (mouse and rat)	<1 nM (mouse)	~1 nM (mouse and rat)
Binds prekallikrein?	No	No	No
Specific inhibitor with respect to fXIa, plasmin, and trypsin	Yes	Yes	Yes
Inhibits bradykinin generation	Yes	Yes	Yes

[a]When M142-H08 was produced as an IgG it was determined to be cleaved in the CDR3 of its heavy chain (GGLLLWFR-ELKSNYFDY).

Example 3

Sequence Optimization of M142-H08

Of the antibodies listed in Table 3, only M142-H08 inhibits human pKal with a subnanomolar $K_{i,app}$. However, when M142-H08 was produced as an IgG it was found to be cleaved in the CDR3 of the heavy chain. M142-H08 was found by mass spectrometry to be cleaved after the arginine in the "WFR" sequence of the HC-CDR3 sequence (GGLLLWFRELKSNYFDY). This cleavage suggests that a protease from the cells used to express the antibody (both CHO and 293T human kidney cells) is enzymatically cleaving the antibody at a single specific site. We mutated the HC-CDR3 sequence of M142-H08 in order to identify amino acid substitutions that prevent the cleavage of the IgG that occurs while retaining the binding and inhibitor properties of M142-H08. Previous experience with similarly "clipped" antibodies suggested that focusing simply on the putative P1 position (protease subsite 1, see Table 4) may not be sufficient to identify antibodies that retain potent inhibition of the target enzyme while not being clipped by a host cell protease. Therefore, we created a small library of single point mutations in the region around the cleavage site in order to identify variants of M142-H08 that are not clipped but are still potent pKal inhibitors. We refer to this library as the "CDR3 by Design" library. The small library was constructed using a PCR primer that contains the randomized codon NNK at either the P3, the P2, the P1, or the P1' site. This results in a small library where each of the 4 positions may contain any of the 20 amino acids (20+20+20+20=80 members). Using PCR, this library was cloned into the M142-H08 Fab sequence in the pMid21 vector, which is a standard phagemid vector.

TABLE 4

Primer sequences

Primer Name	Sequence	N
	P3 P2 P1 P1' P2' G G L L L W F R E L K S N Y	
559A.P1.top	GGC GGT CTA TTA CTA TGG TTC **NNK** GAG CTG AAG TCT AAC TAC	20
559A.P2.top	GGC GGT CTA TTA CTA TGG **NNK** AGG GAG CTG AAG TCT AAC TAC	20
559A.P3.top	GGC GGT CTA TTA CTA **NNK** TTC AGG GAG CTG AAG TCT AAC TAC	20
559A.P1p.top	GGC GGT CTA TTA CTA TGG TTC AGG **NNK** CTG AAG TCT AAC TAC	20

By DNA sequencing, we recovered 61 of the possible 80 antibodies (Table 5). These antibodies were produced as Fab fragments in small scale (~20 μg) and tested for inhibition against human pKal in an in vitro protease cleavage assay using Pro-Phe-Arg-aminomethylcoumarin as the synthetic peptide substrate. The Fabs that were found to be inhibitors of human pKal were subcloned into our pBRH1f vector (a vector for transient expression of IgGs in 293T cells) for conversion to full length human IgG1 antibodies. Five antibodies were then expressed in 293T cells and purified by protein A sepharose chromatography. The antibodies were analyzed by SDS-PAGE to determine which of the inhibitory mutants are not cleaved by the host cell protease(s) (data not shown). The cleaved antibodies (559A-X67-G05, 559A-X67-H01, 559A-X67-G09) had an extra band that migrated between the 38 and the 49 kDa molecular weight marker. This band is absent in the 559A-X67-H04 and 559A-X67-D03 antibodies, which indicates that these antibodies are intact.

$K_{i,app}$ values were determined by steady state enzyme kinetics for those that were shown by SDS-PAGE to be not cleaved (Table 5). Interestingly, the P2 position was the only position where amino acid substitutions yielded intact antibody inhibitors of pKal. Of the 14 different mutations that were recovered at the P3 position (Table 5), only one mutant (W to L) was found to be a pKal inhibitor as a Fab but it was subsequently shown to be clipped as an IgG. None of the 16 different mutations at the P1 position (Table 5) were found to be pKal inhibitors. Eight of the 15 different mutations at the P1' position were found to be inhibitors of pKal as a Fab but all were clipped as an IgG. Consequently, only mutations at the P2 position led to antibody inhibitors that were not clipped during expression. Of the 16 different mutations that were recovered at the P2 position (Table 5), eight mutants were found to be a pKal inhibitor as a Fab but it was subsequently shown to be clipped as an IgG. Four mutants at the P2 position were found to have subnanomolar $K_{i,app}$ values: X67-G04 (F to A), X67-C03 (F to M), X67-F01 (F to Q) and X67-D03 (F to N). The antibody with the highest potency is X67-D03 ($K_{i,app}$=0.1 nM). The two antibodies shown in Table 6 were not cleaved when expressed as IgGs and were found to inhibit pKal with a subnanomolar $K_{i,app}$.

DNA and amino acid sequence alignments of the light chains of nongermlined (X63-G06) and germlined, codon optimized (X81-B01) versions of the same antibody discovered using ROLIC affinity maturation are shown in FIGS. **4** and **5**, respectively. DNA and amino acid sequence alignments of the heavy chains of nongermlined (X63-G06) and germlined, codon optimized (X81-B01) versions of the same antibody discovered using ROLIC affinity maturation are shown in FIGS. **6** and **7**, respectively.

TABLE 5

HV-CDR3 Sequences Obtained from "CDR3 by Design" Library*

Mutation Site	Antibody I.D.	HV-CDR3	Inhibit as a Fab?	Intact as an IgG?	Ki, app as an IgG (nM)
Parental	X69-C09	GGLLL**WFRE**LKSNYFDY	Yes	No	0.2
P3	X68-E07	GGLLL**A**FRELKSNYFDY	No	n/a	n/a
P3	X68-E12	GGLLL**C**FRELKSNYFDY	No	n/a	n/a
P3	X68-A03	GGLLL**D**FRELKSNYFDY	No	n/a	n/a
P3	X68-E03	GGLLL**E**FRELKSNYFDY	No	n/a	n/a
P3	X68-A12	GGLLL**G**FRELKSNYFDY	No	n/a	n/a
P3	X68-D11	GGLLL**K**FRELKSNYFDY	No	n/a	n/a
P3	X68-E01	GGLLL**L**FRELKSNYFDY	Yes	No	n/a
P3	X68-F05	GGLLL**M**FRELKSNYFDY	No	n/a	n/a
P3	X68-D10	GGLLL**P**FRELKSNYFDY	No	n/a	n/a
P3	X68-F10	GGLLL**Q**FRELKSNYFDY	No	n/a	n/a
P3	X68-G01	GGLLL**R**FRELKSNYFDY	No	n/a	n/a
P3	X68-G05	GGLLL**S**FRELKSNYFDY	No	n/a	n/a
P3	X68-F12	GGLLL**T**FRELKSNYFDY	No	n/a	n/a
P3	X68-H04	GGLLL**V**FRELKSNYFDY	No	n/a	n/a
P2	X67-G04	GGLLLW**A**RELKSNYFDY	Yes	Yes	0.35
P2	X67-G01	GGLLLW**C**RELKSNYFDY	No	n/a	n/a
P2	X67-E04	GGLLLW**D**RELKSNYFDY	Yes	Yes	1.3
P2	X67-H04	GGLLLW**E**RELKSNYFDY	Yes	Yes	3.6
P2	X67-C09	GGLLLW**G**RELKSNYFDY	Yes	Yes	8.6
P2	X67-B04	GGLLLW**K**RELKSNYFDY	Yes	No	n/a

TABLE 5-continued

HV-CDR3 Sequences Obtained from "CDR3 by Design" Library*

Mutation Site	Antibody I.D.	HV-CDR3	Inhibit as a Fab?	Intact as an IgG?	Ki, app as an IgG (nM)
P2	X67-G09	GGLLLW**L**RELKSNYFDY	Yes	No	n/a
P2	X67-C03	GGLLLW**M**RELKSNYFDY	Yes	Yes	0.7
P2	X67-D03	GGLLLW**N**RELKSNYFDY	Yes	Yes	0.1
P2	X67-B05	GGLLLW**P**RELKSNYFDY	No	n/a	n/a
P2	X67-F01	GGLLLW**Q**RELKSNYFDY	Yes	Yes	0.9
P2	X67-G05	GGLLLW**R**RELKSNYFDY	Yes	No	n/a
P2	X67-B03	GGLLLW**S**RELKSNYFDY	Yes	Yes	2.1
P2	X67-F10	GGLLLW**T**RELKSNYFDY	Yes	Yes	1.3
P2	X67-H01	GGLLLW**W**RELKSNYFDY	Yes	No	n/a
P2	X67-F08	GGLLLW**Y**RELKSNYFDY	Yes	No	n/a
P1	X66-E09	GGLLLWF**A**ELKSNYFDY	No	n/a	n/a
P1	X66-B05	GGLLLWF**C**ELKSNYFDY	No	n/a	n/a
P1	X66-D03	GGLLLWF**E**ELKSNYFDY	No	n/a	n/a
P1	X66-H04	GGLLLWF**F**ELKSNYFDY	No	n/a	n/a
P1	X66-H02	GGLLLWF**G**ELKSNYFDY	No	n/a	n/a
P1	X66-C11	GGLLLWF**H**ELKSNYFDY	No	n/a	n/a
P1	X66-A07	GGLLLWF**K**ELKSNYFDY	No	n/a	n/a
P1	X66-C03	GGLLLWF**L**ELKSNYFDY	No	n/a	n/a
P1	X66-G05	GGLLLWF**M**ELKSNYFDY	No	n/a	n/a
P1	X66-F10	GGLLLWF**P**ELKSNYFDY	No	n/a	n/a
P1	X66-E04	GGLLLWF**Q**ELKSNYFDY	No	n/a	n/a
P1	X66-F01	GGLLLWF**S**ELKSNYFDY	No	n/a	n/a
P1	X66-H11	GGLLLWF**T**ELKSNYFDY	No	n/a	n/a
P1	X66-C02	GGLLLWF**V**ELKSNYFDY	No	n/a	n/a
P1	X66-F09	GGLLLWF**W**ELKSNYFDY	No	n/a	n/a
P1	X66-G08	GGLLLWF**Y**ELKSNYFDY	No	n/a	n/a
P1'	X69-D08	GGLLLWFR**A**LKSNYFDY	No	n/a	n/a
P1'	X69-B02	GGLLLWFR**C**LKSNYFDY	No	n/a	n/a
P1'	X69-D09	GGLLLWFR**G**LKSNYFDY	Yes	No	n/a
P1'	X69-D02	GGLLLWFR**H**LKSNYFDY	No	n/a	n/a
P1'	X69-A12	GGLLLWFR**K**LKSNYFDY	No	n/a	n/a
P1'	X69-F05	GGLLLWFR**L**LKSNYFDY	Yes	No	n/a
P1'	X69-B08	GGLLLWFR**N**LKSNYFDY	Yes	No	n/a
P1'	X69-A10	GGLLLWFR**P**LKSNYFDY	No	n/a	n/a
P1'	X69-A09	GGLLLWFR**Q**LKSNYFDY	Yes	No	n/a
P1'	X69-E05	GGLLLWFR**R**LKSNYFDY	No	n/a	n/a
P1'	X69-F09	GGLLLWFR**S**LKSNYFDY	Yes	No	n/a

TABLE 5-continued

HV-CDR3 Sequences Obtained from "CDR3 by Design" Library*

Mutation Site	Antibody I.D.	HV-CDR3	Inhibit as a Fab?	Intact as an IgG?	Ki, app as an IgG (nM)
P1'	X69-F01	GGLLLWFR**T**LKSNYFDY	Yes	No	n/a
P1'	X69-C12	GGLLLWFR**V**LKSNYFDY	Yes	No	n/a
P1'	X69-E01	GGLLLWFR**W**LKSNYFDY	Yes	No	n/a
P1'	X69-H10	GGLLLWFR**Y**LKSNYFDY	No	n/a	n/a

*All of these antibodies are single point mutations of the M142-H08 sequence.

Amino Acid Sequences of Light Chain (LC) and Heavy Chain (HC) Variable Domain of pKal Antibodies with Designed HC CDR3s are Shown Below.

```
X68-E07 LC
QDIQMTQSPS SLSAFVGDRV TITCRASQPI DNYLNWYHQK PGKAPKLLIY AASRLQSGVP   60
SRLSGSGFGT DFTLTISSLQ PEDFGNYYCQ QSYTVPYTFG GGTKVEIR               108

X68-E07 HC
EVQLLESGGG LVQPGGSLRL SCAASGFTFS AYSMIWVRQA PGKGLEWVSY IRPSGGRTTY   60
ADSVKGRFTI SRDNSKNTLY LQMNSLRAED TAVYYCARGG LLLAFRELKS NYFDYWGQGT  120
LVTVSSASTK GPSVFPLAPS SKS                                          143

X68-E12 LC
QDIQMTQSPS SLSAFVGDRV TITCRASQPI DNYLNWYHQK PGKAPKLLIY AASRLQSGVP   60
SRLSGSGFGT DFTLTISSLQ PEDFGNYYCQ QSYTVPYTFG GGTKVEIR               108

X68-E12 HC
EVQLLESGGG LVQPGGSLRL SCAASGFTFS AYSMIWVRQA PGKGLEWVSY IRPSGGRTTY   60
ADSVKGRFTI SRDNSKNTLY LQMNSLRAED TAVYYCARGG LLLCFRELKS NYFDYWGQGT  120
LVTVSSASTK GPSVFPLAPS SKS                                          143

X68-A03 LC
QDIQMTQSPS SLSAFVGDRV TITCRASQPI DNYLNWYHQK PGKAPKLLIY AASRLQSGVP   60
SRLSGSGFGT DFTLTISSLQ PEDFGNYYCQ QSYTVPYTFG GGTKVEIR               108

X68-A03 HC
EVQLLESGGG LVQPGGSLRL SCAASGFTFS AYSMIWVRQA PGKGLEWVSY IRPSGGRTTY   60
ADSVKGRFTI SRDNSKNTLY LQMNSLRAED TAVYYCARGG LLLDFRELKS NYFDYWGQGT  120
LVTVSSASTK GPSVFPLAPS SKS                                          143

X68-E03 LC
QDIQMTQSPS SLSAFVGDRV TITCRASQPI DNYLNWYHQK PGKAPKLLIY AASRLQSGVP   60
SRLSGSGFGT DFTLTISSLQ PEDFGNYYCQ QSYTVPYTFG GGTKVEIR               108

X68-E03 HC
EVQLLESGGG LVQPGGSLRL SCAASGFTFS AYSMIWVRQA PGKGLEWVSY IRPSGGRTTY   60
ADSVKGRFTI SRDNSKNTLY LQMNSLRAED TAVYYCARGG LLLEFRELKS NYFDYWGQGT  120
LVTVSSASTK GPSVFPLAPS SKS                                          143

X68-A12 LC
QDIQMTQSPS SLSAFVGDRV TITCRASQPI DNYLNWYHQK PGKAPKLLIY AASRLQSGVP   60
SRLSGSGFGT DFTLTISSLQ PEDFGNYYCQ QSYTVPYTFG GGTKVEIR               108

X68-A12 HC
EVQLLESGGG LVQPGGSLRL SCAASGFTFS AYSMIWVRQA PGKGLEWVSY IRPSGGRTTY   60
ADSVKGRFTI SRDNSKNTLY LQMNSLRAED TAVYYCARGG LLLGFRELKS NYFDYWGQGT  120
LVTVSSASTK GPSVFPLAPS SKS                                          143

X68-D11 LC
QDIQMTQSPS SLSAFVGDRV TITCRASQPI DNYLNWYHQK PGKAPKLLIY AASRLQSGVP   60
SRLSGSGFGT DFTLTISSLQ PEDFGNYYCQ QSYTVPYTFG GGTKVEIR               108

X68-D11 HC
EVQLLESGGG LVQPGGSLRL SCAASGFTFS AYSMIWVRQA PGKGLEWVSY IRPSGGRTTY   60
ADSVKGRFTI SRDNSKNTLY LQMNSLRAED TAVYYCARGG LLLKFRELKS NYFDYWGQGT  120
LVTVSSASTK GPSVFPLAPS SKS                                          143

X68-E01 LC
QDIQMTQSPS SLSAFVGDRV TITCRASQPI DNYLNWYHQK PGKAPKLLIY AASRLQSGVP   60
SRLSGSGFGT DFTLTISSLQ PEDFGNYYCQ QSYTVPYTFG GGTKVEIR               108
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X68-E01 HC
EVQLLESGGG LVQPGGSLRL SCAASGFTFS AYSMIWVRQA PGKGLEWVSY IRPSGGRTTY   60
ADSVKGRFTI SRDNSKNTLY LQMNSLRAED TAVYYCARGG LLLLFRELKS NYFDYWGQGT  120
LVTVSSASTK GPSVFPLAPS SKS                                          143

X68-F05 LC
QDIQMTQSPS SLSAFVGDRV TITCRASQPI DNYLNWYHQK PGKAPKLLIY AASRLQSGVP   60
SRLSGSGFGT DFTLTISSLQ PEDFGNYYCQ QSYTVPYTFG GGTKVEIR               108

X68-F05 HC
EVQLLESGGG LVQPGGSLRL SCAASGFTFS AYSMIWVRQA PGKGLEWVSY IRPSGGRTTY   60
ADSVKGRFTI SRDNSKNTLY LQMNSLRAED TAVYYCARGG LLLMFRELKS NYFDYWGQGT  120
LVTVSSASTK GPSVFPLAPS SKS                                          143

X68-D10 LC
QDIQMTQSPS SLSAFVGDRV TITCRASQPI DNYLNWYHQK PGKAPKLLIY AASRLQSGVP   60
SRLSGSGFGT DFTLTISSLQ PEDFGNYYCQ QSYTVPYTFG GGTKVEIR               108

X68-D10 HC
EVQLLESGGG LVQPGGSLRL SCAASGFTFS AYSMIWVRQA PGKGLEWVSY IRPSGGRTTY   60
ADSVKGRFTI SRDNSKNTLY LQMNSLRAED TAVYYCARGG LLLPFRELKS NYFDYWGQGT  120
LVTVSSASTK GPSVFPLAPS SKS                                          143

X68-F10 LC
QDIQMTQSPS SLSAFVGDRV TITCRASQPI DNYLNWYHQK PGKAPKLLIY AASRLQSGVP   60
SRLSGSGFGT DFTLTISSLQ PEDFGNYYCQ QSYTVPYTFG GGTKVEIR               108

X68-F10 HC
EVQLLESGGG LVQPGGSLRL SCAASGFTFS AYSMIWVRQA PGKGLEWVSY IRPSGGRTTY   60
ADSVKGRFTI SRDNSKNTLY LQMNSLRAED TAVYYCARGG LLLQFRELKS NYFDYWGQGT  120
LVTVSSASTK GPSVFPLAPS SKS                                          143

X68-G01 LC
QDIQMTQSPS SLSAFVGDRV TITCRASQPI DNYLNWYHQK PGKAPKLLIY AASRLQSGVP   60
SRLSGSGFGT DFTLTISSLQ PEDFGNYYCQ QSYTVPYTFG GGTKVEIR               108

X68-G01 HC
EVQLLESGGG LVQPGGSLRL SCAASGFTFS AYSMIWVRQA PGKGLEWVSY IRPSGGRTTY   60
ADSVKGRFTI SRDNSKNTLY LQMNSLRAED TAVYYCARGG LLLRFRELKS NYFDYWGQGT  120
LVTVSSASTK GPSVFPLAPS SKS                                          143

X68-G05 LC
QDIQMTQSPS SLSAFVGDRV TITCRASQPI DNYLNWYHQK PGKAPKLLIY AASRLQSGVP   60
SRLSGSGFGT DFTLTISSLQ PEDFGNYYCQ QSYTVPYTFG GGTKVEIR               108

X68-G05 HC
EVQLLESGGG LVQPGGSLRL SCAASGFTFS AYSMIWVRQA PGKGLEWVSY IRPSGGRTTY   60
ADSVKGRFTI SRDNSKNILY LQMNSLRAED TAVYYCARGG LLLSFRELKS NYFDYWGQGT  120
LVTVSSASTK GPSVFPLAPS SKS                                          143

X68-F12 LC
QDIQMTQSPS SLSAFVGDRV TITCRASQPI DNYLNWYHQK PGKAPKLLIY AASRLQSGVP   60
SRLSGSGFGT DFTLTISSLQ PEDFGNYYCQ QSYTVPYTFG GGTKVEIR               108

X68-F12 HC
EVQLLESGGG LVQPGGSLRL SCAASGFTFS AYSMIWVRQA PGKGLEWVSY IRPSGGRTTY   60
ADSVKGRFTI SRDNSKNTLY LQMNSLRAED TAVYYCARGG LLLTFRELKS NYFDYWGQGT  120
LVTVSSASTK GPSVFPLAPS SKS                                          143

X68-H04 LC
QDIQMTQSPS SLSAFVGDRV TITCRASQPI DNYLNWYHQK PGKAPKLLIY AASRLQSGVP   60
SRLSGSGFGT DFTLTISSLQ PEDFGNYYCQ QSYTVPYTFG GGTKVEIR               108

X68-H04 HC
EVQLLESGGG LVQPGGSLRL SCAASGFTFS AYSMIWVRQA PGKGLEWVSY IRPSGGRTTY   60
ADSVKGRFTI SRDNSKNTLY LQMNSLRAED TAVYYCARGG LLLVFRELKS NYFDYWGQGT  120
LVTVSSASTK GPSVFPLAPS SKS                                          143

X67-G04 LC
QDIQMTQSPS SLSAFVGDRV TITCRASQPI DNYLNWYHQK PGKAPKLLIY AASRLQSGVP   60
SRLSGSGFGT DFTLTISSLQ PEDFGNYYCQ QSYTVPYTFG GGTKVEIR               108

X67-G04 HC
EVQLLESGGG LVQPGGSLRL SCAASGFTFS AYSMIWVRQA PGKGLEWVSY IRPSGGRTTY   60
ADSVKGRFTI SRDNSKNTLY LQMNSLRAED TAVYYCARGG LLLWARELKS NYFDYWGQGT  120
LVTVSSASTK GPSVFPLAPS SKS                                          143

X67-G01 LC
QDIQMTQSPS SLSAFVGDRV TITCRASQPI DNYLNWYHQK PGKAPKLLIY AASRLQSGVP   60
SRLSGSGFGT DFTLTISSLQ PEDFGNYYCQ QSYTVPYTFG GGTKVEIR               108
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X67-G01 HC
EVQLLESGGG LVQPGGSLRL SCAASGFTFS AYSMIWVRQA PGKGLEWVSY IRPSGGRTTY 60
ADSVKGRFTI SRDNSKNTLY LQMNSLRAED TAVYYCARGG LLLWCRELKS NYFDYWGQGT 120
LVTVSSASTK GPSVFPLAPS SKS 143

X67-E04 LC
QDIQMTQSPS SLSAFVGDRV TITCRASQPI DNYLNWYHQK PGKAPKLLIY AASRLQSGVP 60
SRLSGSGFGT DFTLTISSLQ PEDFGNYYCQ QSYTVPYTFG GGTKVEIR 108

X67-E04 HC
EVQLLESGGG LVQPGGSLRL SCAASGFTFS AYSMIWVRQA PGKGLEWVSY IRPSGGRTTY 60
ADSVKGRFTI SRDNSKNTLY LQMNSLRAED TAVYYCARGG LLLWDRELKS NYFDYWGQGT 120
LVTVSSASTK GPSVFPLAPS SKS 143

X67-H04 LC
QDIQMTQSPS SLSAFVGDRV TITCRASQPI DNYLNWYHQK PGKAPKLLIY AASRLQSGVP 60
SRLSGSGFGT DFTLTISSLQ PEDFGNYYCQ QSYTVPYTFG GGTKVEIR 108

X67-H04 HC
EVQLLESGGG LVQPGGSLRL SCAASGFTFS AYSMIWVRQA PGKGLEWVSY IRPSGGRTTY 60
ADSVKGRFTI SRDNSKNTLY LQMNSLRAED TAVYYCARGG LLLWERELKS NYFDYWGQGT 120
LVTVSSASTK GPSVFPLAPS SKS 143

X66-E09 LC
QDIQMTQSPS SLSAFVGDRV TITCRASQPI DNYLNWYHQK PGKAPKLLIY AASRLQSGVP 60
SRLSGSGFGT DFTLTISSLQ PEDFGNYYCQ QSYTVPYTFG GGTKVEIR 108

X66-E09 HC
EVQLLESGGG LVQPGGSLRL SCAASGFTFS AYSMIWVRQA PGKGLEWVSY IRPSGGRTTY 60
ADSVKGRFTI SRDNSKNTLY LQMNSLRAED TAVYYCARGG LLLWFAELKS NYFDYWGQGT 120
LVTVSSASTK GPSVFPLAPS SKS 143

X66-B05 LC
QDIQMTQSPS SLSAFVGDRV TITCRASQPI DNYLNWYHQK PGKAPKLLIY AASRLQSGVP 60
SRLSGSGFGT DFTLTISSLQ PEDFGNYYCQ QSYTVPYTFG GGTKVEIR 108

X66-B05 HC
EVQLLESGGG LVQPGGSLRL SCAASGFTFS AYSMIWVRQA PGKGLEWVSY IRPSGGRTTY 60
ADSVKGRFTI SRDNSKNTLY LQMNSLRAED TAVYYCARGG LLLWFCELKS NYFDYWGQGT 120
LVTVSSASTK GPSVFPLAPS SKS 143

X66-D03 LC
QDIQMTQSPS SLSAFVGDRV TITCRASQPI DNYLNWYHQK PGKAPKLLIY AASRLQSGVP 60
SRLSGSGFGT DFTLTISSLQ PEDFGNYYCQ QSYTVPYTFG GGTKVEIR 108

X66-D03 HC
EVQLLESGGG LVQPGGSLRL SCAASGFTFS AYSMIWVRQA PGKGLEWVSY IRPSGGRTTY 60
ADSVKGRFTI SRDNSKNTLY LQMNSLRAED TAVYYCARGG LLLWFEELKS NYFDYWGQGT 120
LVTVSSASTK GPSVFPLAPS SKS 143

X66-H04 LC
QDIQMTQSPS SLSAFVGDRV TITCRASQPI DNYLNWYHQK PGKAPKLLIY AASRLQSGVP 60
SRLSGSGFGT DFTLTISSLQ PEDFGNYYCQ QSYTVPYTFG GGTKVEIR 108

X66-H04 HC
EVQLLESGGG LVQPGGSLRL SCAASGFTFS AYSMIWVRQA PGKGLEWVSY IRPSGGRTTY 60
ADSVKGRFTI SRDNSKNTLY LQMNSLRAED TAVYYCARGG LLLWFFELKS NYFDYWGQGT 120
LVTVSSASTK GPSVFPLAPS SKS 143

X66-H02 LC
QDIQMTQSPS SLSAFVGDRV TITCRASQPI DNYLNWYHQK PGKAPKLLIY AASRLQSGVP 60
SRLSGSGFGT DFTLTISSLQ PEDFGNYYCQ QSYTVPYTFG GGTKVEIR 108

X66-H02 HC
EVQLLESGGG LVQPGGSLRL SCAASGFTFS AYSMIWVRQA PGKGLEWVSY IRPSGGRTTY 60
ADSVKGRFTI SRDNSKNTLY LQMHSLRAED TAVYYCARGG LLLWFGELKS NYFDYWGQGT 120
LVTVSSASTK GPSVFPLAPS SKS 143

X66-C11 LC
QDIQMTQSPS SLSAFVGDRV TITCRASQPI DNYLNWYHQK PGKAPKLLIY AASRLQSGVP 60
SRLSGSGFGT DFTLTISSLQ PEDFGNYYCQ QSYTVPYTFG GGTKVEIR 108

X66-C11 HC
EVQLLESGGG LVQPGGSLRL SCAASGFTFS AYSMIWVRQA PGKGLEWVSY IRPSGGRTTY 60
ADSVKGRFTI SRDNSKNTLY LQMNSLRAED TAVYYCARGG LLLWFHELKS NYFDYWGQGT 120
LVTVSSASTK GPSVFPLAPS SKS 143

X66-A07 LC
QDIQMTQSPS SLSAFVGDRV TITCRASQPI DNYLNWYHQK PGKAPKLLIY AASRLQSGVP 60
SRLSGSGFGT DFTLTISSLQ PEDFGNYYCQ QSYTVPYTFG GGTKVEIR 108

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X66-A07 HC
EVQLLESGGG LVQPGGSLRL SCAASGFTFS AYSMIWVRQA PGKGLEWVSY IRPSGGRTTY 60
ADSVKGRFTI SRDNSKNTLY LQMNSLRAED TAVYYCARGG LLLWFKELKS NYFDYWGQGT 120
LVTVSSASTK GPSVFPLAPS SKS 143

X66-C03 LC
QDIQMTQSPS SLSAFVGDRV TITCRASQPI DNYLNWYHQK PGKAPKLLIY AASRLQSGVP 60
SRLSGSGFGT DFTLTISSLQ PEDFGNYYCQ QSYTVPYTFG GGTKVEIR 108

X66-C03 HC
EVQLLESGGG LVQPGGSLRL SCAASGFTFS AYSMIWVRQA PGKGLEWVSY IRPSGGRTTY 60
ADSVKGRFTI SRDNSKNTLY LQMNSLRAED TAVYYCARGG LLLWFLELKS NYFDYWGQGT 120
LVTVSSASTK GPSVFPLAPS SKS 143

X66-G05 LC
QDIQMTQSPS SLSAFVGDRV TITCRASQPI DNYLNWYHQK PGKAPKLLIY AASRLQSGVP 60
SRLSGSGFGT DFTLTISSLQ PEDFGNYYCQ QSYTVPYTFG GGTKVEIR 108

X66-G05 HC
EVQLLESGGG LVQPGGSLRL SCAASGFTFS AYSMIWVRQA PGKGLEWVSY IRPSGGRTTY 60
ADSVKGRFTI SRDNSKNTLY LQMNSLRAED TAVYYCARGG LLLWFMELKS NYFDYWGQGT 120
LVTVSSASTK GPSVFPLAPS SKS 143

X66-F10 LC
QDIQMTQSPS SLSAFVGDRV TITCRASQPI DNYLNWYHQK PGKAPKLLIY AASRLQSGVP 60
SRLSGSGFGT DFTLTISSLQ PEDFGNYYCQ QSYTVPYTFG GGTKVEIR 108

X66-F10 HC
EVQLLESGGG LVQPGGSLRL SCAASGFTFS AYSMIWVRQA PGKGLEWVSY IRPSGGRTTY 60
ADSVKGRFTI SRDNSKNTLY LQMNSLRAED TAVYYCARGG LLLWFPELKS NYFDYWGQGT 120
LVTVSSASTK GPSVFPLAPS SKS 143

X66-E04 LC
QDIQMTQSPS SLSAFVGDRV TITCRASQPI DNYLNWYHQK PGKAPKLLIY AASRLQSGVP 60
SRLSGSGFGT DFTLTISSLQ PEDFGNYYCQ QSYTVPYTFG GGTKVEIR 108

X66-E04 HC
EVQLLESGGG LVQPGGSLRL SCAASGFTFS AYSMIWVRQA PGKGLEWVSY IRPSGGRTTY 60
ADSVKGRFTI SRDNSKNTLY LQMNSLRAED TAVYYCARGG LLLWFQELKS NYFDYWGQGT 120
LVTVSSASTK GPSVFPLAPS SKS 143

X69-D08 LC
QDIQMTQSPS SLSAFVGDRV TITCRASQPI DNYLNWYHQK PGKAPKLLIY AASRLQSGVP 60
SRLSGSGFGT DFTLTISSLQ PEDFGNYYCQ QSYTVPYTFG GGTKVEIR 108

X69-D08 HC
EVQLLESGGG LVQPGGSLRL SCAASGFTFS AYSMIWVRQA PGKGLEWVSY IRPSGGRTTY 60
ADSVKGRFTI SRDNSKNTLY LQMNSLRAED TAVYYCARGG LLLWFRALKS NYFDYWGQGT 120
LVTVSSASTK GPSVFPLAPS SKS 143

X69-B02 LC
QDIQMTQSPS SLSAFVGDRV TITCRASQPI DNYLNWYHQK PGKAPKLLIY AASRLQSGVP 60
SRLSGSGFGT DFTLTISSLQ PEDFGNYYCQ QSYTVPYTFG GGTKVEIR 108

X69-B02 HC
EVQLLESGGG LVQPGGSLRL SCAASGFTFS AYSMIWVRQA PGKGLEWVSY IRPSGGRTTY 60
ADSVKGRFTI SRDNSKNTLY LQMNSLRAED TAVYYCARGG LLLWFRCLKS NYFDYWGQGT 120
LVTVSSASTK GPSVFPLAPS SKS 143

X69-009 LC
QDIQMTQSPS SLSAFVGDRV TITCRASQPI DNYLNWYHQK PGKAPKLLIY AASRLQSGVP 60
SRLSGSGFGT DFTLTISSLQ PEDFGNYYCQ QSYTVPYTFG GGTKVEIR 108

X69-009 HC
EVQLLESGGG LVQPGGSLRL SCAASGFTFS AYSMIWVRQA PGKGLEWVSY IRPSGGRTTY 60
ADSVKGRFTI SRDNSKNTLY LQMNSLRAED TAVYYCARGG LLLWFRELKS NYFDYWGQGT 120
LVTVSSASTK GPSVFPLAPS SKS 143

X69-D09 LC
QDIQMTQSPS SLSAFVGDRV TITCRASQPI DNYLNWYHQK PGKAPKLLIY AASRLQSGVP 60
SRLSGSGFGT DFTLTISSLQ PEDFGNYYCQ QSYTVPYTFG GGTKVEIR 108

X69-D09 HC
EVQLLESGGG LVQPGGSLRL SCAASGFTFS AYSMIWVRQA PGKGLEWVSY IRPSGGRTTY 60
ADSVKGRFTI SRDNSKNTLY LQMNSLRAED TAVYYCARGG LLLWFRGLKS NYFDYWGQGT 120
LVTVSSASTK GPSVFPLAPS SKS 143

X69-D02 LC
QDIQMTQSPS SLSAFVGDRV TITCRASQPI DNYLNWYHQK PGKAPKLLIY AASRLQSGVP 60
SRLSGSGFGT DFTLTISSLQ PEDFGNYYCQ QSYTVPYTFG GGTKVEIR 108

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X69-D02 HC
EVQLLESGGG LVQPGGSLRL SCAASGFTFS AYSMIWVRQA PGKGLEWVSY IRPSGGRTTY 60
ADSVKGRFTI SRDNSKNTLY LQMNSLRAED TAVYYCARGG LLLWFRHLKS NYFDYWGQGT 120
LVTVSSASTK GPSVFPLAPS SKS 143

X69-A12 LC
QDIQMTQSPS SLSAFVGDRV TITCRASQPI DNYLNWYHQK PGKAPKLLIY AASRLQSGVP 60
SRLSGSGFGT DFTLTISSLQ PEDFGNYYCQ QSYTVPYTFG GGTKVEIR 108

X69-A12 HC
EVQLLESGGG LVQPGGSLRL SCAASGFTFS AYSMIWVRQA PGKGLEWVSY IRPSGGRTTY 60
ADSVKGRFTI SRDNSKNTLY LQMNSLRAED TAVYYCARGG LLLWFRKLKS NYFDYWGQGT 120
LVTVSSASTKG PSVFPLAPS SKS 143

X69-F05 LC
QDIQMTQSPS SLSAFVGDRV TITCRASQPI DNYLNWYHQK PGKAPKLLIY AASRLQSGVP 60
SRLSGSGFGT DFTLTISSLQ PEDFGNYYCQ QSYTVPYTFG GGTKVEIR 108

X69-F05 HC
EVQLLESGGG LVQPGGSLRL SCAASGFTFS AYSMIWVRQA PGKGLEWVSY IRPSGGRTTY 60
ADSVKGRFTI SRDNSKNTLY LQMNSLRAED TAVYYCARGG LLLWFRLLKS NYFDYWGQGT 120
LVTVSSASTK GPSVFPLAPS SKS 143

X69-B08 LC
QDIQMTQSPS SLSAFVGDRV TITCRASQPI DNYLNWYHQK PGKAPKLLIY AASRLQSGVP 60
SRLSGSGFGT DFTLTISSLQ PEDFGNYYCQ QSYTVPYTFG GGTKVEIR 108

X69-B08 HC
EVQLLESGGG LVQPGGSLRL SCAASGFTFS AYSMIWVRQA PGKGLEWVSY IRPSGGRTTY 60
ADSVKGRFTI SRDNSKNTLY LQMNSLRAED TAVYYCARGG LLLWFRNLKS NYFDYWGQGT 120
LVTVSSASTK GPSVFPLAPS SKS 143

X69-A10 LC
QDIQMTQSPS SLSAFVGDRV TITCRASQPI DNYLNWYHQK PGKAPKLLIY AASRLQSGVP 60
SRLSGSGFGT DFTLTISSLQ PEDFGNYYCQ QSYTVPYTFG GGTKVEIR 108

X69-A10 HC
EVQLLESGGG LVQPGGSLRL SCAASGFTFS AYSMIWVRQA PGKGLEWVSY IRPSGGRTTY 60
ADSVKGRFTI SRDNSKNTLY LQMNSLRAED TAVYYCARGG LLLWFRPLKS NYFDYWGQGT 120
LVTVSSASTK GPSVFPLAPS SKS 143

X69-A09 LC
QDIQMTQSPS SLSAFVGDRV TITCRASQPI DNYLNWYHQK PGKAPKLLIY AASRLQSGVP 60
SRLSGSGFGT DFTLTISSLQ PEDFGNYYCQ QSYTVPYTFG GGTKVEIR 108

X69-A09 HC
EVQLLESGGG LVQPGGSLRL SCAASGFTFS AYSMIWVRQA PGKGLEWVSY IRPSGGRTTY 60
ADSVKGRFTI SRDNSKNTLY LQMNSLRAED TAVYYCARGG LLLWFRQLKS NYFDYWGQGT 120
LVTVSSASTK GPSVFPLAPS SKS 143

X69-E05 LC
QDIQMTQSPS SLSAFVGDRV TITCRASQPI DNYLNWYHQK PGKAPKLLIY AASRLQSGVP 60
SRLSGSGFGT DFTLTISSLQ PEDFGNYYCQ QSYTVPYTFG GGTKVEIR 108

X69-E05 HC
EVQLLESGGG LVQPGGSLRL SCAASGFTFS AYSMIWVRQA PGKGLEWVSY IRPSGGRTTY 60
ADSVKGRFTI SRDNSKNTLY LQMNSLRAED TAVYYCARGG LLLWFRRLKS NYFDYWGQGT 120
LVTVSSASTK GPSVFPLAPS SKS 143

X69-F09 LC
QDIQMTQSPS SLSAFVGDRV TITCRASQPI DNYLNWYHQK PGKAPKLLIY AASRLQSGVP 60
SRLSGSGFGT DFTLTISSLQ PEDFGNYYCQ QSYTVPYTFG GGTKVEIR 108

X69-F09 HC
EVQLLESGGG LVQPGGSLRL SCAASGFTFS AYSMIWVRQA PGKGLEWVSY IRPSGGRTTY 60
ADSVKGRFTI SRDNSKNTLY LQMNSLRAED TAVYYCARGG LLLWFRSLKS NYFDYWGQGT 120
LVTVSSASTK GPSVFPLAPS SKS 143

X69-F01 LC
QDIQMTQSPS SLSAFVGDRV TITCRASQPI DNYLNWYHQK PGKAPKLLIY AASRLQSGVP 60
SRLSGSGFGT DFTLTISSLQ PEDFGNYYCQ QSYTVPYTFG GGTKVEIR 108

X69-F01 HC
EVQLLESGGG LVQPGGSLRL SCAASGFTFS AYSMIWVRQA PGKGLEWVSY IRPSGGRTTY 60
ADSVKGRFTI SRDNSKNTLY LQMNSLRAED TAVYYCARGG LLLWFRTLKS NYFDYWGQGT 120
LVTVSSASTK GPSVFPLAPS SKS 143

X69-C12 LC
QDIQMTQSPS SLSAFVGDRV TITCRASQPI DNYLNWYHQK PGKAPKLLIY AASRLQSGVP 60
SRLSGSGFGT DFTLTISSLQ PEDFGNYYCQ QSYTVPYTFG GGTKVEIR 108

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X69-C12 HC
EVQLLESGGG LVQPGGSLRL SCAASGFTFS AYSMIWVRQA PGKGLEWVSY IRPSGGRTTY   60
ADSVKGRFTI SRDNSKNTLY LQMNSLRAED TAVYYCARGG LLLWFRVLKS NYFDYWGQGT  120
LVTVSSASTK GPSVFPLAPS SKS                                          143

X69-E01 LC
QDIQMTQSPS SLSAFVGDRV TITCRASQPI DNYLNWYHQK PGKAPKLLIY AASRLQSGVP   60
SRLSGSGFGT DFTLTISSLQ PEDFGNYYCQ QSYTVPYTFG GGTKVEIR               108

X69-E01 HC
EVQLLESGGG LVQPGGSLRL SCAASGFTFS AYSMIWVRQA PGKGLEWVSY IRPSGGRTTY   60
ADSVKGRFTI SRDNSKNTLY LQMNSLRAED TAVYYCARGG LLLWFRWLKS NYFDYWGQGT  120
LVTVSSASTK GPSVFPLAPS SKS                                          143

X69-H10 LC
QDIQMTQSPS SLSAFVGDRV TITCRASQPI DNYLNWYHQK PGKAPKLLIY AASRLQSGVP   60
SRLSGSGFGT DFTLTISSLQ PEDFGNYYCQ QSYTVPYTFG GGTKVEIR               108

X69-H10 HC
EVQLLESGGG LVQPGGSLRL SCAASGFTFS AYSMIWVRQA PGKGLEWVSY IRPSGGRTTY   60
ADSVKGRFTI SRDNSKNTLY LQMNSLRAED TAVYYCARGG LLLWFRYLKS NYFDYWGQGT  120
LVTVSSASTK GPSVFPLAPS SKS                                          143

X66-F01 LC
QDIQMTQSPS SLSAFVGDRV TITCRASQPI DNYLNWYHQK PGKAPKLLIY AASRLQSGVP   60
SRLSGSGFGT DFTLTISSLQ PEDFGNYYCQ QSYTVPYTFG GGTKVEIR               108

X66-F01 HC
EVQLLESGGG LVQPGGSLRL SCAASGFTFS AYSMIWVRQA PGKGLEWVSY IRPSGGRTTY   60
ADSVKGRFTI SRDNSKNTLY LQMNSLRAED TAVYYCARGG LLLWFSELKS NYFDYWGQGT  120
LVTVSSASTK GPSVFPLAPS SKS                                          143

X66-H11 LC
QDIQMTQSPS SLSAFVGDRV TITCRASQPI DNYLNWYHQK PGKAPKLLIY AASRLQSGVP   60
SRLSGSGFGT DFTLTISSLQ PEDFGNYYCQ QSYTVPYTFG GGTKVEIR               108

X66-H11 HC
EVQLLESGGG LVQPGGSLRL SCAASGFTFS AYSMIWVRQA PGKGLEWVSY IRPSGGRTTY   60
ADSVKGRFTI SRDNSKNTLY LQMNSLRAED TAVYYCARGG LLLWFTELKS NYFDYWGQGT  120
LVTVSSASTK GPSVFPLAPS SKS                                          143

X66-C02 LC
QDIQMTQSPS SLSAFVGDRV TITCRASQPI DNYLNWYHQK PGKAPKLLIY AASRLQSGVP   60
SRLSGSGFGT DFTLTISSLQ PEDFGNYYCQ QSYTVPYTFG GGTKVEIR               108

X66-C02 HC
EVQLLESGGG LVQPGGSLRL SCAASGFTFS AYSMIWVRQA PGKGLEWVSY IRPSGGRTTY   60
ADSVKGRFTI SRDNSKNTLY LQMNSLRAED TAVYYCARGG LLLWFVELKS NYFDYWGQGT  120
LVTVSSASTK GPSVFPLAPS SKS                                          143

X66-F09 LC
QDIQMTQSPS SLSAFVGDRV TITCRASQPI DNYLNWYHQK PGKAPKLLIY AASRLQSGVP   60
SRLSGSGFGT DFTLTISSLQ PEDFGNYYCQ QSYTVPYTFG GGTKVEIR               108

X66-F09 HC
EVQLLESGGG LVQPGGSLRL SCAASGFTFS AYSMIWVRQA PGKGLEWVSY IRPSGGRTTY   60
ADSVKGRFTI SRDNSKNTLY LQMNSLRAED TAVYYCARGG LLLWFWELKS NYFDYWGQGT  120
LVTVSSASTK GPSVFPLAPS SKS                                          143

X66-G08 LC
QDIQMTQSPS SLSAFVGDRV TITCRASQPI DNYLNWYHQK PGKAPKLLIY AASRLQSGVP   60
SRLSGSGFGT DFTLTISSLQ PEDFGNYYCQ QSYTVPYTFG GGTKVEIR               108

X66-G08 HC
EVQLLESGGG LVQPGGSLRL SCAASGFTFS AYSMIWVRQA PGKGLEWVSY IRPSGGRTTY   60
ADSVKGRFTI SRDNSKNTLY LQMNSLRAED TAVYYCARGG LLLWFYELKS NYFDYWGQGT  120
LVTVSSASTK GPSVFPLAPS SKS                                          143

X67-C09 LC
QDIQMTQSPS SLSAFVGDRV TITCRASQPI DNYLNWYHQK PGKAPKLLIY AASRLQSGVP   60
SRLSGSGFGT DFTLTISSLQ PEDFGNYYCQ QSYTVPYTFG GGTKVEIR               108

X67-C09 HC
EVQLLESGGG LVQPGGSLRL SCAASGFTFS AYSMIWVRQA PGKGLEWVSY IRPSGGRTTY   60
ADSVKGRFTI SRDNSKNTLY LQMNSLRAED TAVYYCARGG LLLWGRELKS NYFDYWGQGT  120
LVTVSSASTK GPSVFPLAPS SKS                                          143

X67-B04 LC
QDIQMTQSPS SLSAFVGDRV TITCRASQPI DNYLNWYHQK PGKAPKLLIY AASRLQSGVP   60
SRLSGSGFGT DFTLTISSLQ PEDFGNYYCQ QSYTVPYTFG GGTKVEIR               108
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X67-B04 HC
EVQLLESGGG LVQPGGSLRL SCAASGFTFS AYSMIWVRQA PGKGLEWVSY IRPSGGRTTY 60
ADSVKGRFTI SRDNSKNTLY LQMNSLRAED TAVYYCARGG LLLWKRELKS NYFDYWGQGT 120
LVTVSSASTK GPSVFPLAPS SKS 143

X67-G09 LC
QDIQMTQSPS SLSAFVGDRV TITCRASQPI DNYLNWYHQK PGKAPKLLIY AASRLQSGVP 60
SRLSGSGFGT DFTLTISSLQ PEDFGNYYCQ QSYTVPYTFG GGTKVEIR 108

X67-G09 HC
EVQLLESGGG LVQPGGSLRL SCAASGFTFS AYSMIWVRQA PGKGLEWVSY IRPSGGRTTY 60
ADSVKGRFTI SRDNSKNTLY LQMNSLRAED TAVYYCARGG LLLWLRELKS NYFDYWGQGT 120
LVTVSSASTK GPSVFPLAPS SKS 143

X67-C03 LC
QDIQMTQSPS SLSAFVGDRV TITCRASQPI DNYLNWYHQK PGKAPKLLIY AASRLQSGVP 60
SRLSGSGFGT DFTLTISSLQ PEDFGNYYCQ QSYTVPYTFG GGTKVEIR 108

X67-C03 HC
EVQLLESGGG LVQPGGSLRL SCAASGFTFS AYSMIWVRQA PGKGLEWVSY IRPSGGRTTY 60
ADSVKGRFTI SRDNSKNTLY LQMNSLRAED TAVYYCARGG LLLWMRELKS NYFDYWGQGT 120
LVTVSSASTK GPSVFPLAPS SKS 143

X67-D03 LC
QDIQMTQSPS SLSAFVGDRV TITCRASQPI DNYLNWYHQK PGKAPKLLIY AASRLQSGVP 60
SRLSGSGFGT DFTLTISSLQ PEDFGNYYCQ QSYTVPYTFG GGTKVEIR 108

X67-D03 HC
EVQLLESGGG LVQPGGSLRL SCAASGFTFS AYSMIWVRQA PGKGLEWVSY IRPSGGRTTY 60
ADSVKGRFTI SRDNSKNTLY LQMNSLRAED TAVYYCARGG LLLWNRELKS NYFDYWGQGT 120
LVTVSSASTK GPSVFPLAPS SKS 143

X67-B05 LC
QDIQMTQSPS SLSAFVGDRV TITCRASQPI DNYLNWYHQK PGKAPKLLIY AASRLQSGVP 60
SRLSGSGFGT DFTLTISSLQ PEDFGNYYCQ QSYTVPYTFG GGTKVEIR 108

X67-B05 HC
EVQLLESGGG LVQPGGSLRL SCAASGFTFS AYSMIWVRQA PGKGLEWVSY IRPSGGRTTY 60
ADSVKGRFTI SRDNSKNTLY LQMNSLRAED TAVYYCARGG LLLWPRELKS NYFDYWGQGT 120
LVTVSSASTK GPSVFPLAPS SKS 143

X67-F01 LC
QDIQMTQSPS SLSAFVGDRV TITCRASQPI DNYLNWYHQK PGKAPKLLIY AASRLQSGVP 60
SRLSGSGFGT DFTLTISSLQ PEDFGNYYCQ QSYTVPYTFG GGTKVEIR 108

X67-F01 HC
EVQLLESGGG LVQPGGSLRL SCAASGFTFS AYSMIWVRQA PGKGLEWVSY IRPSGGRTTY 60
ADSVKGRFTI SRDNSKNTLY LQMNSLRAED TAVYYCARGG LLLWQRELKS NYFDYWGQGT 120
LVTVSSASTK GPSVFPLAPS SKS 143

X67-G05 LC
QDIQMTQSPS SLSAFVGDRV TITCRASQPI DNYLNWYHQK PGKAPKLLIY AASRLQSGVP 60
SRLSGSGFGT DFTLTISSLQ PEDFGNYYCQ QSYTVPYTFG GGTKVEIR 108

X67-G05 HC
EVQLLESGGG LVQPGGSLRL SCAASGFTFS AYSMIWVRQA PGKGLEWVSY IRPSGGRTTY 60
ADSVKGRFTI SRDNSKNTLY LQMNSLRAED TAVYYCARGG LLLWRRELKS NYFDYWGQGT 120
LVTVSSASTK GPSVFPLAPS SKS 143

X67-B03 LC
QDIQMTQSPS SLSAFVGDRV TITCRASQPI DNYLNWYHQK PGKAPKLLIY AASRLQSGVP 60
SRLSGSGFGT DFTLTISSLQ PEDFGNYYCQ QSYTVPYTFG GGTKVEIR 108

X67-B03 HC
EVQLLESGGG LVQPGGSLRL SCAASGFTFS AYSMIWVRQA PGKGLEWVSY IRPSGGRTTY 60
ADSVKGRFTI SRDNSKNTLY LQMNSLRAED TAVYYCARGG LLLWSRELKS NYFDYWGQGT 120
LVTVSSASTK GPSVFPLAPS SKS 143

X67-F10 LC
QDIQMTQSPS SLSAFVGDRV TITCRASQPI DNYLNWYHQK PGKAPKLLIY AASRLQSGVP 60
SRLSGSGFGT DFTLTISSLQ PEDFGNYYCQ QSYTVPYTFG GGTKVEIR 108

X67-F10 HC
EVQLLESGGG LVQPGGSLRL SCAASGFTFS AYSMIWVRQA PGKGLEWVSY IRPSGGRTTY 60
ADSVKGRFTI SRDNSKNTLY LQMNSLRAED TAVYYCARGG LLLWTRELKS NYFDYWGQGT 120
LVTVSSASTK GPSVFPLAPS SKS 143

X67-H01 LC
QDIQMTQSPS SLSAFVGDRV TITCRASQPI DNYLNWYHQK PGKAPKLLIY AASRLQSGVP 60
SRLSGSGFGT DFTLTISSLQ PEDFGNYYCQ QSYTVPYTFG GGTKVEIR 108

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X67-H01 HC
EVQLLESGGG LVQPGGSLRL SCAASGFTFS AYSMIWVRQA PGKGLEWVSY IRPSGGRTTY   60
ADSVKGRFTI SRDNSKNTLY LQMNSLRAED TAVYYCARGG LLLWWRELKS NYFDYWGQGT  120
LVTVSSASTK GPSVFPLAPS SKS                                           143

X67-F08 LC
QDIQMTQSPS SLSAFVGDRV TITCRASQPI DNYLNWYHQK PGKAPKLLIY AASRLQSGVP   60
SRLSGSGFGT DFTLTISSLQ PEDFGNYYCQ QSYTVPYTFG GGTKVEIR                108

X67-F08 HC
EVQLLESGGG LVQPGGSLRL SCAASGFTFS AYSMIWVRQA PGKGLEWVSY IRPSGGRTTY   60
ADSVKGRFTI SRDNSKNTLY LQMNSLRAED TAVYYCARGG LLLWYRELKS NYFDYWGQGT  120
LVTVSSASTK GPSVFPLAPS SKS                                           143
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TABLE 6

CDR Amino Acid Sequences of Optimized Antibody Inhibitor of pKal Based on M142-1108

Initial Name	Ki, app (nM) of IgG	LV-CDR1	LV-CDR2	LV-CDR3	HV-CDR1	HV-CDR2	HV-CDR3[a]
X67-D03	0.1	RASQPIDNYLN	AASRLQS	QQSYTVPYT	AYSMI	YIRPSGGRTTYADSVKG	GGLLLWW**N**RELKSNYFDY
X67-G04	0.35	RASQPIDNYLN	AASRLQS	QQSYTVPYT	AYSMI	YIRPSGGRTTYADSVKG	GGLLLWA**A**RELKSNYFDY

[a]The F to N substitution (in bold) in the CDR3 of the M142-H08 gives X67-D03 an IgG that is not cleaved during expression and is a potent inhibitor of human. Similarly, the F to A substitution gives X67-G04, which is also not cleaved.

TABLE 7

CDR Amino Acid Sequences of Affinity Matured Antibody Inhibitors of pKal Discovered using ROLIC

Initial Name	Ki, app (nM)	LV-CDR1	LV-CDR2	LV-CDR3	HV-CDR1	HV-CDR2	HV-CDR3
X59-C07	6.1	RAGRSISTYVN	AASSLQS	QQSQSTPYT	HYLMT	YISPSGGHTIYADSVKG	VARGIAARSRTSYFDY
X60-D01	2.0	RASQIVSSRYLA	GAASRAT	QQTYSSPFT	HYLMT	YISPSGGHTIYADSVKG	VARGIAARSRTSYFDY
X63-G10	9.0	RASQSISNYLN	AASSLQS	QQSYTSPYT	HYLMT	YISPSGGHTIYADSVKG	VARGIAARSRTSYFDY
X64-F04	1.9	RASQIVSSNYLA	GASNRAT	QQSFNIPYT	HYLMT	YISPSGGHTIYADSVKG	VARGIAARSRTSYFDY
X63-G06	0.4 (Fab)	RTSQFVNSNYLA	GASSRAT	QQSSRTPWT	HYLMT	YISPSGGHTIYADSVKG	VARGIAARSRTSYFDY
X81-B01[a]	0.2 (IgG)	RTSQFVNSNYLA	GASSRAT	QQSSRTPWT	HYLMT	YISPSGGHTIYADSVKG	VARGIAARSRTSYFDY

[a]X81-B01 is the codon optimized and germlined version of X63-G06 as a full length human IgG produced in HEK 293T cells.

Amino Acid Sequences of Light Chain (LC) and Heavy Chain (HC) Variable Domain of Affinity Matured Antibody Inhibitors of pKal Discovered Using ROLIC are Shown Below.

```
X59-C07 LC
QDIQMTQSPS SLSASVGDRV TVTCRAGRSI STYVNWYQQK PGKAPKLLIY AASSLQSGVP   60
SRFSGSRSGT DFTLTISSLQ PEDFATYYCQ QSQSTPYTFG QGTKLEVK                108

X59-C07 HC
EVQLLESGGG LVQPGGSLRL SCAASGFTFS HYLMTWVRQA PGKGLEWVSY ISPSGGHTIY   60
ADSVKGRFTI SRDNSKNTLY LQMNSLRAED TAVYYCARVA RGIAARSRTS YFDYWGQGTL  120
VTVSSASTKG PSVFPLAPSS KS                                            142

X60-D01 LC
QDIQMTQSPG TLSLSPGERA TLSCRASQIV SSRYLAWYQQ RPGQAPRLLI YGAASRATGI   60
PDRFSGSGSG TDFTLTISSL QAEDFATYYC QQTYSSPFTF GQGTKMEIK               109

X60-D01 HC
EVQLLESGGG LVQPGGSLRL SCAASGFTFS HYLMTWVRQA PGKGLEWVSY ISPSGGHTIY   60
ADSVKGRFTI SRDNSKNTLY LQMNSLRAED TAVYYCARVA RGIAARSRTS YFDYWGQGTL  120
VTVSSASTKG PSVFPLAPSS KS                                            142
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X63-G06 LC
QDIQMTQSPG TLSLSPGERA TLSCRTSQFV NSNYLAWYQQ TPGQAPRLLI YGASSRATGI   60
PDRFSGTGYG TDFTLTISRL EPEDYGTYYC QQSSRTPWTF GQGTRVEIK              109

X63-G06 HC
EVQLLESGGG LVQPGGSLRL SCAASGFTFS HYLMTWVRQA PGKGLEWVSY ISPSGGHTIY   60
ADSVKGRFTI SRDNSKNTLY LQMNSLRAED TAVYYCARVA RGIAARSRTS YFDYWGQGTL  120
VTVSSASTKG PSVFPLAPSS KS                                           142

X63-G10 LC
QDIQMTQSPD SLSASVGDRV TITCRASQSI SNYLNWYQQK PGKAPKLLIY AASSLQSGVP   60
SRFSGSGSGT DFTLTISGLQ PEDFASYYCQ QSYTSPYTFV QGTKLEIKRT             110

X63-G10 HC
EVQLLESGGG LVQPGGSLRL SCAASGFTFS HYLMTWVRQA PGKGLEWVSY ISPSGGHTIY   60
ADSVKGRFTI SRDNSKNTLY LQMNSLRAED TAVYYCARVA RGIAARSRTS YFDYWGQGTL  120
VTVSSASTKG PSVFPLAPSS KS                                           142

X64-F04 LC
QDIQMTQSPA TLSLSPGERA TLSCRASQIV SSNYLAWYQQ KPGQAPRLLI YGASNRATGI   60
PDRFSGSGSG TEFTLTISSL QSEDFAIYYC QQSFNIPYTF GQGTRVDIK              109

X64-F04 HC
EVQLLESGGG LVQPGGSLRL SCAASGFTFS HYLMTWVRQA PGKGLEWVSY ISPSGGHTIY   60
ADSVKGRFTI SRDNSKNTLY LQMNSLRAED TAVYYCARVA RGIAARSRTS YFDYWGQGTL  120
VTVSSASTKG PSVFPLAPSS KS                                           142
```

X81-B01 is the germlined IgG produced in HEK 293T cells version of the X63-G06 Fab, as indicated above.

X101-A01 (aka DX-2922) is the germlined IgG produced in CHO cells version of the X63-G06 Fab

Example 4

Affinity Maturation

In addition to optimizing the sequence of the clipped antibody (M142-H08), we also performed affinity maturation on two of the antibodies identified by phage display (M162-A04 and M160-G12). Both of these antibodies inhibit human pKal with single digit nanomolar potency, appear specific to pKal, and do not bind prekallikrein (Table 3). We first performed a novel form of light chain shuffling called ROLIC (Rapid Optimization of Light Chains) on M162-A04 and M160-G12 (see, e.g., WO 2009/102927 and U.S. 2009-0215119). From the screening of the antibodies discovered by ROLIC we identified one antibody with subnamolar potency (X63-G06) that shared the same heavy chain as M160-G12. We then constructed HV-CDR3 spiking affinity maturation libraries based on CDR3 sequences in M162-A04 and X63-G06 (described below).

Affinity Maturation by ROLIC.

We used ROLIC to affinity mature the two leads from Table 3 that were not cleaved (M162-A04 and M160-G12). This process identified one antibody that inhibits pKal with a subnanomolar $K_{i,app}$ (Table 7). X63-G06 inhibits pKal with a $K_{i,app}$ of approximately 0.4 nM as a Fab fragment. When this antibody was converted to an IgG that is germlined and sequenced optimized for CHO cell expression (X81-B01) it was found to inhibit pKal with a $K_{i,app}$ of approximately 0.2 nM.

Example 5

Affinity Maturation of Heavy Chain CDR1/2 and CDR3

We used two additional affinity maturation strategies to identify highly potent antibodies based on two different parental antibody inhibitor leads: M162-A04 and X63-G06. One approach was to generate libraries that shuffled the CDR1/2 of the HC of two different parental antibody inhibitor leads (M162-A04 and X63-G06) against additional CDR1/2 diversity. Another approach was to create heavy chain CDR3 spiking libraries based on these leads.

The 82 antibodies that were discovered based on improvements in M162-A04 due to modifications in either the CDR1/2 and CDR3 region are shown in Table 8. Inhibition screening with 10 nM antibody (as Fab fragments) revealed that there were 33 antibodies that inhibited pKal activity by over 90%. Several antibodies were shown to be subnanomolar inhibitors of human pKal.

The 62 antibodies that were discovered based on improvements in X63-G06 due to modifications in either the CDR1/2 and CDR3 region are shown in Table 9. Inhibition screening with 10 nM antibody (as Fab fragments) revealed that there were 24 antibodies that inhibited pKal activity by over 90%. Several antibodies were shown to be subnanomolar inhibitors of human pKal.

TABLE 8

Sequences of Antibodies Obtained from CDR1/2 and CDR3 Spiking Affinity Maturation Libraries Based on M162-A04

Antibody I.D.	% inhibition at 10 nM	human pKal Ki, app (nM)	LV-CDR1	LV-CDR2	LV-CDR3	HV-CDR1	HV-CDR2	HV-CDR3
M202-A12	97.5	0.2	RASQSISSWLA	KASTLES	QQYNTYWT	HYIMM	GIYSSGGITVYADSVKG	QRTGVPRRDSFNI
M196-C06	97.2	0.1	RASQSISSWLA	KASTLES	QQYNTYWT	IYSMH	SIYPSRGMTWYADSVKG	RRTGIPRRDAFDI

TABLE 8-continued

Sequences of Antibodies Obtained from CDR1/2 and CDR3 Spiking Affinity Maturation Libraries Based on M162-A04

Antibody I.D.	% inhibition at 10 nM	human pKal Ki, app (nM)	LV-CDR1	LV-CDR2	LV-CDR3	HV-CDR1	HV-CDR2	HV-CDR3
M198-F09	96.9	0.2	RASQSISSWLA	KASTLES	QQYNTYWT	VYNMH	SIYPSGGMTYYADSVKG	RRTGIPRRDAFDI
M199-A08	96.4	0.06	RASQSISSWLA	KASTLES	QQYNTYWT	HYIMM	GIYSSGGITVYADSVKG	RRIGVPRRDEFDI
M202-C01	96.3	0.1	RASQSISSWLA	KASTLES	QQYNTYWT	HYIMM	GIYSSGGITVYADSVKG	RRTGVPRWDDFDI
M198-A06	96.1	0.4	RASQSISSWLA	KASTLES	QQYNTYWT	IYSMH	SIYSSGGPTKYADSVKG	RRTGIPRRDAFDI
M200-D03	95.9	0.1	RASQSISSWLA	KASTLES	QQYNTYWT	HYIMM	GIYSSGGITVYADSVKG	RRIGVPRRDSFDM
M202-H03	95.7	0.1	RASQSISSWLA	KASTLES	QQYNTYWT	HYIMM	GIYSSGGITVYADSVKG	RRTGVPRWDDFDI
M201-A07	95.7	0.1	RASQSISSWLA	KASTLES	QQYNTYWT	HYIMM	GIYSSGGITVYADSVKG	RRTGVPRRDEFDI
M197-A01	95.3		RASQSISSWLA	KASTLES	QQYNTYWT	IYDMI	SIYPSGGNTSYADSVKG	RRTGIPRRDAFDI
M202-D09	95.0	0.4	RASQSISSWLA	KASTLES	QQYNTYWT	HYIMM	GIYSSGGITVYADSVKG	RRIGVPRRDSFDI
M197-A09	94.9	0.6	RASQSISSWLA	KASTLES	QQYNTYWT	VYNMH	SIYPSGGMTTYADSVKG	RRTGIPRRDAFDI
M198-G07	94.9		RASQSISSWLA	KASTLES	QQYNTYWT	IYDMT	SIYPSGGQTIYADSVKG	RRTGIPRRDAFDI
M200-A10	94.3	0.3	RASQSISSWLA	KASTLES	QQYNTYWT	HYIMM	GIYSSGGITVYADSVKG	RRTGVPRRDSFDI
M197-H10	94.1		RASQSISSWLA	KASTLES	QQYNTYWT	SYNMH	SIVPSGGKTNYADSVKG	RRTGIPRRDAFDI
M196-D12	94.1	0.2	RASQSISSWLA	KASTLES	QQYNTYWT	RYSMR	VIYPSGGQTYYADSVKG	RRTGIPRRDAFDI
M197-A08	93.7		RASQSISSWLA	KASTLES	QQYNTYWT	IYSMQ	SIGSSGGKTLYADSVKG	RRTGIPRRDAFDI
M198-B09	93.5		RASQSISSWLA	KASTLES	QQYNTYWT	VYSMT	SIGSSGGSTTYADSVKG	RRTGIPRRDAFDI
M198-E09	93.1		RASQSISSWLA	KASTLES	QQYNTYWT	IYDMN	SIYPSGGRTRYADSVKG	RRTGIPRRDAFDI
M202-B03	93.1	0.3	RASQSISSWLA	KASTLES	QQYNTYWT	HYIMM	GIYSSGGITVYADSVKG	RRTGVPRRDDFDI
M198-C10	93.0		RASQSISSWLA	KASTLES	QQYNTYWT	HYMGMN	SIVPSGGWTQYADSVKG	RRTGIPRRDAFDI
M197-E12	93.0		RASQSISSWLA	KASTLES	QQYNTYWT	TYTMR	SIYPSGGKTQYADSVKG	RRTGIPRRDAFDI
M198-F04	92.9		RASQSISSWLA	KASTLES	QQYNTYWT	IYDMW	SIRPSGGITKYADSVKG	RRTGIPRRDAFDI
M197-H11	92.9		RASQSISSWLA	KASTLES	QQYNTYWT	IYNMI	SIYPSGGWTTYADSVKG	RRTGIPRRDAFDI
M197-F01	92.6		RASQSISSWLA	KASTLES	QQYNTYWT	IYHMY	SIGPSGGPTGYADSVKG	RRTGIPRRDAFDI
M198-E11	92.5		RASQSISSWLA	KASTLES	QQYNTYWT	TYSMY	SIYPSGGLTWYADSVKG	RRTGIPRRDAFDI
M202-C09	92.3	0.3	RASQSISSWLA	KASTLES	QQYNTYWT	HYIMM	GIYSSGGITVYADSVKG	RRIGVPRRDDFDI
M198-H08	92.3		RASQSISSWLA	KASTLES	QQYNTYWT	IYDMY	SIGPSGGPTAYADSVKG	RRTGIPRRDAFDI
M198-F08	91.8		RASQSISSWLA	KASTLES	QQYNTYWT	VYSMW	SISSSGGMTEYADSVKG	RRTGIPRRDAFDI
M202-E06	91.5		RASQSISSWLA	KASTLES	QQYNTYWT	HYIMM	GIYSSGGITVYADSVKG	RRRGVPRRDDFDI
M195-D12	90.8		RASQSISSWLA	KASTLES	QQYNTYWT	IYGMF	GIGPSGGPTKYADSVKG	RRTGIPRRDAFDI
M197-F03	90.7		RASQSISSWLA	KASTLES	QQYNTYWT	IYSMF	SIGPSGGVTHYADSVKG	RRTGIPRRDAFDI
M198-E02	90.3		RASQSISSWLA	KASTLES	QQYNTYWT	IYSMY	YIRPSGGNTKYADSVKG	RRTGIPRRDAFDI
M198-A02	89.1		RASQSISSWLA	KASTLES	QQYNTYWT	RYSMI	SIWSSGGATEYADSVKG	RRTGIPRRDAFDI
M202-A01	88.9		RASQSISSWLA	KASTLES	QQYNTYWT	HYIMM	GIYSSGGITVYADSVKG	RRIGVPRRDAFDI
M202-G03	88.3		RASQSISSWLA	KASTLES	QQYNTYWT	HYIMM	GIYSSGGITVYADSVKG	RRTGVPRRDSFEI
M195-B12	87.7		RASQSISSWLA	KASTLES	QQYNTYWT	KYWMY	YIRPSGGQTYYADSVKG	RRTGIPRRDAFDI
M198-A07	86.1		RASQSISSWLA	KASTLES	QQYNTYWT	RYQMH	WISPSGGITGYADSVKG	RRTGIPRRDAFDI

TABLE 8-continued

Sequences of Antibodies Obtained from CDR1/2 and CDR3 Spiking Affinity Maturation Libraries Based on M162-A04

Antibody I.D.	% inhibition at 10 nM	human pKal Ki, app (nM)	LV-CDR1	LV-CDR2	LV-CDR3	HV-CDR1	HV-CDR2	HV-CDR3
M198-H02	85.8		RASQSISSWLA	KASTLES	QQYNTYWT	PYNMY	WIVPGGVTKYADSVKG	RRTGIPRRDAFDI
M200-H07	85.4		RASQSISSWLA	KASTLES	QQYNTYWT	HYIMM	GIYSSGGITVYADSVKG	RRTGVPRRNAFDN
M201-H06	84.6		RASQSISSWLA	KASTLES	QQYNTYWT	HYIMM	GIYSSGGITVYADSVKG	RRTGVPRRDAFDI
M202-F06	84.2		RASQSISSWLA	KASTLES	QQYNTYWT	HYIMM	GIYSSGGITVYADSVKG	RRTGVPRWDAFDI
M195-C12	84.2		RASQSISSWLA	KASTLES	QQYNTYWT	MYQMF	SISPGGGTQYADSVKG	RRTGIPRRDAFDI
M202-H05	84.0		RASQSISSWLA	KASTLES	QQYNTYWT	HYIMM	GIYSSGGITVYADSVKG	RRTGVPRRDVFDI
M198-C05	83.9		RASQSISSWLA	KASTLES	QQYNTYWT	RYKMY	VIGPSGGATFYADSVKG	RRTGIPRRDAFDI
M196-H03	83.9		RASQSISSWLA	KASTLES	QQYNTYWT	RYVMW	SISPSGDTHYADSVKG	RRTGIPRRDAFDI
M200-E11	83.2		RASQSISSWLA	KASTLES	QQYNTYWT	HYIMM	GIYSSGGITVYADSVKG	RRTGVPRRDAFDN
M202-B04	81.9		RASQSISSWLA	KASTLES	QQYNTYWT	HYIMM	GIYSSGGITVYADSVKG	RRSGVPRRDDFDI
M202-A04	81.2		RASQSISSWLA	KASTLES	QQYNTYWT	HYIMM	GIYSSGGITVYADSVKG	RRKGIPRRDDFDI
M198-B12	80.7		RASQSISSWLA	KASTLES	QQYNTYWT	KYSMA	GIYPSGGRTLYADSVKG	RRTGIPRRDAFDI
M198-A09	77.3		RASQSISSWLA	KASTLES	QQYNTYWT	IYFMS	SIRSSGGPTWYADSVKG	RRTGIPRRDAFDI
M198-C06	76.5		RASQSISSWLA	KASTLES	QQYNTYWT	QYFMH	YIYPSGGMTEYADSVKG	RRTGIPRRDAFDI
M198-C09	75.4		RASQSISSWLA	KASTLES	QQYNTYWT	IYTMY	SISPSGGWTYYADSVKG	RRTGIPRRDAFDI
M195-B02	75.1		RASQSISSWLA	KASTLES	QQYNTYWT	PYLMW	YIGPSGGPTHYADSVKG	RRTGIPRRDAFDI
M198-F12	74.6		RASQSISSWLA	KASTLES	QQYNTYWT	IYTMM	SIWSSGGQTKYADSVKG	RRTGIPRRDAFDI
M201-H08	74.5		RASQSISSWLA	KASTLES	QQYNTYWT	HYIMM	GIYSSGGITVYADSVKG	RRTGVPRRDALDN
M202-C02	74.3		RASQSISSWLA	KASTLES	QQYNTYWT	HYIMM	GIYSSGGITVYADSVKG	RRPGVPRRDAFDI
M198-C03	72.4		RASQSISSWLA	KASTLES	QQYNTYWT	RYSMS	GISPSGGETSYADSVKG	RRTGIPRRDAFDI
M198-A08	72.3		RASQSISSWLA	KASTLES	QQYNTYWT	WYMMQ	RISPSGGTTYADSVKG	RRTGIPRRDAFDI
M195-A02	71.3		RASQSISSWLA	KASTLES	QQYNTYWT	QYMMM	GISSSGGHTDYADSVKG	RRTGIPRRDAFDI
M197-G10	67.6		RASQSISSWLA	KASTLES	QQYNTYWT	VYAMR	SIYPSGGKTWYADSVKG	RRTGIPRRDAFDI
M195-G02	67.5		RASQSISSWLA	KASTLES	QQYNTYWT	PYNMM	SIWPSGGTTDYADSVKG	RRTGIPRRDAFDI
M196-D02	66.2		RASQSISSWLA	KASTLES	QQYNTYWT	VYSMH	VIGPSGGITLYADSVKG	RRTGIPRRDAFDI
M199-A11	65.4		RASQSISSWLA	KASTLES	QQYNTYWT	HYIMM	GIYSSGGITVYADSVKG	RRRGIPRRDAFDI
M200-F01	65.1		RASQSISSWLA	KASTLES	QQYNTYWT	HYIMM	GIYSSGGITVYADSVKG	RRMGIPRRNAFDI
M198-D12	63.5	0.7	RASQSISSWLA	KASTLES	QQYNTYWT	LYVMY	YIVPSGGPTAYADSVKG	RRTGIPRRDAFDI
M197-C12	56.4		RASQSISSWLA	KASTLES	QQYNTYWT	PYDML	YIVSSGGLTKYADSVKG	RRTGIPRRDAFDI
M198-G03	53.8		RASQSISSWLA	KASTLES	QQYNTYWT	QYTMV	WIYSSRANYADSVKG	RRTGIPRRDAFDI
M199-B01	53.4		RASQSISSWLA	KASTLES	QQYNTYWT	HYIMM	GIYSSGGITVYADSVKG	RRTGIPRRDAFDN
M202-A08	52.9		RASQSISSWLA	KASTLES	QQYNTYWT	HYIMM	GIYSSGGITVYADSVKG	RRTGIPRWDAFDI
M195-A12	51.7		RASQSISSWLA	KASTLES	QQYNTYWT	PYMMM	GIYPSGGYTVYADSVKG	RRTGIPRRDAFDI
M202-E03	51.4		RASQSISSWLA	KASTLES	QQYNTYWT	HYIMM	GIYSSGGITVYADSVKG	RRTGIPRRDAFEI
M196-G12	51.1		RASQSISSWLA	KASTLES	QQYNTYWT	NYSMD	RIYSSGGGTIYADSVKG	RRTGIPRRDAFDI
M195-F12	45.5		RASQSISSWLA	KASTLES	QQYNTYWT	HYVMM	YIVPSGGVTAYADSVKG	RRTGIPRRDAFDI

TABLE 8-continued

Sequences of Antibodies Obtained from CDR1/2 and CDR3 Spiking Affinity Maturation Libraries Based on M162-A04

Antibody I.D.	% inhibition at 10 nM	human pKal Ki, app (nM)	LV-CDR1	LV-CDR2	LV-CDR3	HV-CDR1	HV-CDR2	HV-CDR3
M200-B01	42.6		RASQSISSWLA	KASTLES	QQYNTYWT	HYIMM	GIYSSGGITVYADSVKG	RRTGIPRRDAFDS
M198-H09	41.1		RASQSISSWLA	KASTLES	QQYNTYWT	IYLMI	YIGPSGGPTEYADSVKG	RRTGIPRRDAFDI
M195-E12	38.0		RASQSISSWLA	KASTLES	QQYNTYWT	YYIMF	YISPSGGYTHYADSVKG	RRTGIPRRDAFDI
M201-A06	36.8		RASQSISSWLA	KASTLES	QQYNTYWT	HYIMM	GIYSSGGITVYADSVKG	RRTGIPRRDVFDI
M202-A10	36.3		RASQSISSWLA	KASTLES	QQYNTYWT	HYIMM	GIYSSGGITVYADSVKG	RRTGIPRRDSFDI
M197-G11	19.2		RASQSISSWLA	KASTLES	QQYNTYWT	TYAMV	SIYPSGGITTYADSVKG	RRTGIPRRDAFDI
M201-F11	15.7		RASQSISSWLA	KASTLES	QQYNTYWT	HYIMM	GIYSSGGITVYADSVKG	RRSGIPRRDAFDI
M198-A01	13.8		RASQSISSWLA	KASTLES	QQYNTYWT	PYTMI	SISSSGGMTPYADSVKG	RRTGIPRRDAFDI

Amino Acid Sequences of Light Chain (LC) and Heavy Chain (HC) Variable Domain of pKal Antibodies Obtained from CDR1/2 and CDR3 Spiking Affinity Maturation Libraries Based on M162-A04.

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M195-A02                LC
QDIQMTQSPS TLSASVGDRV TITCRASQSI SSWLAWYQQK PGKAPNLLIY KASTLESGVP  60
SRFSGSGSGT EFTLTISSLQ PDDFATYYCQ QYNTYWTFGQ GTKVEIK               107

M195-A02                HC
EVQLLESGGG LVQPGGSLRL SCAASGFTFS QYMMMWVRQA PGKGLEWVSG ISSSGGHTDY  60
ADSVKGRFTI SRDNSKNTLY LQMNSLRAED TAVYYCAYRR TGIPRRDAFD IWGQGTMVTV 120
SSASTKGPSV FPLAPSSKS                                              139

M195-A12                LC
QDIQMTQSPS TLSASVGDRV TITCRASQSI SSWLAWYQQK PGKAPNLLIY KASTLESGVP  60
SRFSGSGSGT EFTLTISSLQ PDDFATYYCQ QYNTYWTFGQ GTKVEIK               107

M195-A12                HC
EVQLLESGGG LVQPGGSLRL SCAASGFTFS PYMMMWVRQA PGKGLEWVSG IYPSGGYTVY  60
ADSVKGRFTI SRDNSKNTLY LQMNSLRAED TAVYYCAYRR TGIPRRDAFD IWGQGTMVTV 120
SSASTKGPSV FPLAPSSKS                                              139

M195-B02                LC
QDIQMTQSPS TLSASVGDRV TITCRASQSI SSWLAWYQQK PGKAPNLLIY KASTLESGVP  60
SRFSGSGSGT EFTLTISSLQ PDDFATYYCQ QYNTYWTFGQ GTKVEIK               107

M195-B02                HC
EVQLLESGGG LVQPGGSLRL SCAASGFTFS PYLMWWVRQA PGKGLEWVSY IGPSGGPTHY  60
ADSVKGRFTI SRDNSKNTLY LQMNSLRAED TAVYYCAYRR TGIPRRDAFD IWGQGTMVTV 120
SSASTKGPSV FPLAPSSKS                                              139

M195-B12                LC
QDIQMTQSPS TLSASVGDRV TITCRASQSI SSWLAWYQQK PGKAPNLLIY KASTLESGVP  60
SRFSGSGSGT EFTLTISSLQ PDDFATYYCQ QYNTYWTFGQ GTKVEIK               107

M195-B12                HC
EVQLLESGGG LVQPGGSLRL SCAASGFTFS KYWMYWVRQA PGKGLEWVSY IRPSGGQTYY  60
ADSVKGRFTI SRDNSKNTLY LQMNSLRAED TAVYYCAYRR TGIPRRDAFD IWGQGTMVTV 120
SSASTKGPSV FPLAPSSKS                                              139

M195-C12                LC
QDIQMTQSPS TLSASVGDRV TITCRASQSI SSWLAWYQQK PGKAPNLLIY KASTLESGVP  60
SRFSGSGSGT EFTLTISSLQ PDDFATYYCQ QYNTYWTFGQ GTKVEIK               107

M195-C12                HC
EVQLLESGGG LVQPGGSLRL SCAASGFTFS MYQMFWVRQA PGKGLEWVSS ISPGGGTQYA  60
DSVKGRFTIS RDNSKNTLYL QMNSLRAEDT AVYYCAYRRT GIPRRDAFDI WGQGTMVTVS 120
SASTKGPSVF PLAPSSKS                                               138
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M195-D12                 LC
QDIQMTQSPS TLSASVGDRV TITCRASQSI SSWLAWYQQK PGKAPNLLIY KASTLESGVP  60
SRFSGSGSGT EFTLTISSLQ PDDFATYYCQ QYNTYWTFGQ GTKVEIK               107

M195-D12                 HC
EVQLLESGGG LVQPGGSLRL SCAASGFTFS IYGMFWVRQA PGKGLEWVSG IGPSGGPTKY  60
ADSVKGRFTI SRDNSKNTLY LQMNSLRAED TAVYYCAYRR TGIPRRDAFD IWGQGTMVTV 120
SSASTKGPSV FPLAPSSKS                                              139

M195-E12                 LC
QDIQMTQSPS TLSASVGDRV TITCRASQSI SSWLAWYQQK PGKAPNLLIY KASTLESGVP  60
SRFSGSGSGT EFTLTISSLQ PDDFATYYCQ QYNTYWTFGQ GTKVEIK               107

M195-E12                 HC
EVQLLESGGG LVQPGGSLRL SCAASGFTFS YYIMFWVRQA PGKGLEWVSY ISPSGGYTHY  60
ADSVKGRFTI SRDNSKNTLY LQMNSLRAED TAVYYCAYRR TGIPRRDAFD IWGQGTMVTV 120
SSASTKGPSV FPLAPSSKS                                              139

M195-F12                 LC
QDIQMTQSPS TLSASVGDRV TITCRASQSI SSWLAWYQQK PGKAPNLLIY KASTLESGVP  60
SRFSGSGSGT EFTLTISSLQ PDDFATYYCQ QYNTYWTFGQ GTKVEIK               107

M195-F12                 HC
EVQLLESGGG LVQPGGSLRL SCAASGFTFS HYVMMWVRQA PGKGLEWVSY IVPSGGVTAY  60
ADSVKGRFTI SRDNSKNTLY LQMNSLRAED TAVYYCAYRR TGIPRRDAFD IWGQGTMVTV 120
SSASTKGPSV FPLAPSSKS                                              139

M0195-G02                 LC
QDIQMTQSPS TLSASVGDRV TITCRASQSI SSWLAWYQQK PGKAPNLLIY KASTLESGVP  60
SRFSGSGSGT EFTLTISSLQ PDDFATYYCQ QYNTYWTFGQ GTKVEIK               107

M195-G02                 HC
EVQLLESGGG LVQPGGSLRL SCAASGFTFS PYNMMWVRQA PGKGLEWVSS IWPSGGTTDY  60
ADSVKGRFTI SRDNSKNTLY LQMNSLRAED TAVYYCAYRR TGIPRRDAFD IWGQGTMVTV 120
SSASTKGPSV FPLAPSSKS                                              139

M196-C06                 LC
QDIQMTQSPS TLSASVGDRV TITCRASQSI SSWLAWYQQK PGKAPNLLIY KASTLESGVP  60
SRFSGSGSGT EFTLTISSLQ PDDFATYYCQ QYNTYWTFGQ GTKVEIK               107

M196-C06                 HC
EVQLLESGGG LVQPGGSLRL SCAASGFTFS IYSMHWVRQA PGKGLEWVSS IYPSRGMTWY  60
ADSVKGRFTI SRDNSKNTLY LQMNSLRAED TAVYYCAYRR TGIPRRDAFD IWGQGTMVTV 120
SSASTKGPSV FPLAPSSKS                                              139

M196-D02                 LC
QDIQMTQSPS TLSASVGDRV TITCRASQSI SSWLAWYQQK PGKAPNLLIY KASTLESGVP  60
SRFSGSGSGT EFTLTISSLQ PDDFATYYCQ QYNTYWTFGQ GTKVEIK               107

M196-D02                 HC
EVQLLESGGG LVQPGGSLRL SCAASGFTFS VYSMHWVRQA PGKGLEWVSV IGPSGGITLY  60
ADSVKGRFTI SRDNSKNTLY LQMNSLRAED TAVYYCAYRR TGIPRRDAFD IWGQGTMVTV 120
SSASTKGPSV FPLAPSSKS                                              139

M196-D12                 LC
QDIQMTQSPS TLSASVGDRV TITCRASQSI SSWLAWYQQK PGKAPNLLIY KASTLESGVP  60
SRFSGSGSGT EFTLTISSLQ PDDFATYYCQ QYNTYWTFGQ GTKVEIK               107

M196-D12                 HC
EVQLLESGGG LVQPGGSLRL SCAASGFTFS RYSMRWVRQA PGKGLEWVSV IYPSGGQTYY  60
ADSVKGRFTI SRDNSKNTLY LQMNSLRAED TAVYYCAYRR TGIPRRDAFD IWGQGTMVTV 120
SSASTKGPSV FPLAPSSKS                                              139

M196-G12                 LC
QDIQMTQSPS TLSASVGDRV TITCRASQSI SSWLAWYQQK PGKAPNLLIY KASTLESGVP  60
SRFSGSGSGT EFTLTISSLQ PDDFATYYCQ QYNTYWTFGQ GTKVEIK               107

M196-G12                 HC
EVQLLESGGG LVQPGGSLRL SCAASGFTFS NYSMDWVRQA PGKGLEWVSR IYSSGGGTIY  60
ADSVKGRFTI SRDNSKNTLY LQMNSLRAED TAVYYCAYRR TGIPRRDAFD IWGQGTMVTV 120
SSASTKGPSV FPLAPSSKS                                              139

M196-H03                 LC
QDIQMTQSPS TLSASVGDRV TITCRASQSI SSWLAWYQQK PGKAPNLLIY KASTLESGVP  60
SRFSGSGSGT EFTLTISSLQ PDDFATYYCQ QYNTYWTFGQ GTKVEIK               107

M196-H03                 HC
EVQLLESGGG LVQPGGSLRL SCAASGFTFS RYVMWWVRQA PGKGLEWVSS ISPSGDTHYA  60
DSVKGRFTIS RDNSKNTLYL QMNSLRAEDT AVYYCAYRRT GIPRRDAFDI WGQGTMVTVS 120
SASTKGPSVF PLAPSSKS                                               138
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M197-A01 LC
QDIQMTQSPS TLSASVGDRV TITCRASQSI SSWLAWYQQK PGKAPNLLIY KASTLESGVP 60
SRFSGSGSGT EFTLTISSLQ PDDFATYYCQ QYNTYWTFGQ GTKVEIK 107

M197-A01 HC
EVQLLESGGG LVQPGGSLRL SCAASGFTFS IYDMIWVRQA PGKGLEWVSS IYPSGGNTSY 60
ADSVKGRFTI SRDNSKNTLY LQMNSLRAED TAVYYCAYRR TGIPRRDAFD IWGQGTMVTV 120
SSASTKGPSV FPLAPSSKS 139

M197-A08 LC
QDIQMTQSPS TLSASVGDRV TITCRASQSI SSWLAWYQQK PGKAPNLLIY KASTLESGVP 60
SRFSGSGSGT EFTLTISSLQ PDDFATYYCQ QYNTYWTFGQ GTKVEIK 107

M197-A08 HC
EVQLLESGGG LVQPGGSLRL SCAASGFTFS IYSMQWVRQA PGKGLEWVSS IGSSGGKTLY 60
ADSVKGRFTI SRDNSKNTLY LQMNSLRAED TAVYYCAYRR TGIPRRDAFD IWGQGTMVTV 130
SSASTKGPSV FPLAPSSKS 139

M197-A09 LC
QDIQMTQSPS TLSASVGDRV TITCRASQSI SSWLAWYQQK PGKAPNLLIY KASTLESGVP 60
SRFSGSGSGT EFTLTISSLQ PDDFATYYCQ QYNTYWTFGQ GTKVEIK 107

M197-A09 HC
EVQLLESGGG LVQPGGSLRL SCAASGFTFS VYNMHWVRQA PGKGLEWVSS IYPSGGMTTY 60
ADSVKGRFTI SRDNSKNTLY LQMNSLRAED TAVYYCAYRR TGIPRRDAFD IWGQGTMVTV 120
SSASTKGPSV FPLAPSSKS 139

M197-C12 LC
QDIQMTQSPS TLSASVGDRV TITCRASQSI SSWLAWYQQK PGKAPNLLIY KASTLESGVP 60
SRFSGSGSGT EFTLTISSLQ PDDFATYYCQ QYNTYWTFGQ GTKVEIK 107

M197-C12 HC
EVQLLESGGG LVQPGGSLRL SCAASGFTFS PYDMLWVRQA PGKGLEWVSY IVSSGGLTKY 60
ADSVKGRFTI SRDNSKNTLY LQMNSLRAED TAVYYCAYRR TGIPRRDAFD IWGQGTMVTV 120
SSASTKGPSV FPLAPSSKS 139

M197-E12 LC
QDIQMTQSPS TLSASVGDRV TITCRASQSI SSWLAWYQQK PGKAPNLLIY KASTLESGVP 60
SRFSGSGSGT EFTLTISSLQ PDDFATYYCQ QYNTYWTFGQ GTKVEIK 107

M197-E12 HC
EVQLLESGGG LVQPGGSLRL SCAASGFTFS TYTMRWVRQA PGKGLEWVSS IYPSGGKTQY 60
ADSVKGRFTI SRDNSKNTLY LQMNSLRAED TAVYYCAYRR TGIPRRDAFD IWGQGTMVTV 120
SSASTKGPSV FPLAPSSKS 139

M197-F01 LC
QDIQMTQSPS TLSASVGDRV TITCRASQSI SSWLAWYQQK PGKAPNLLIY KASTLESGVP 60
SRFSGSGSGT EFTLTISSLQ PDDFATYYCQ QYNTYWTFGQ GTKVEIK 107

M197-F01 HC
EVQLLESGGG LVQPGGSLRL SCAASGFTFS IYHMYWVRQA PGKGLEWVSS IGPSGGPTGY 60
ADSVKGRFTI SRDNSKNTLY LQMNSLRAED TAVYYCAYRR TGIPRRDAFD IWGQGTMVTV 120
SSASTKGPSV FPLAPSSKS 139

M197-F03 LC
QDIQMTQSPS TLSASVGDRV TITCRASQSI SSWLAWYQQK PGKAPNLLIY KASTLESGVP 60
SRFSGSGSGT EFTLTISSLQ PDDFATYYCQ QYNTYWTFGQ GTKVEIK 107

M197-F03 HC
EVQLLESGGG LVQPGGSLRL SCAASGFTFS IYSMFWVRQA PGKGLEWVSS IGPSGGVTHY 60
ADSVKGRFTI SRDNSKNTLY LQMNSLRAED TAVYYCAYRR TGIPRRDAFD IWGQGTMVTV 120
SSASTKGPSV FPLAPSSKS 139

M197-G10 LC
QDIQMTQSPS TLSASVGDRV TITCRASQSI SSWLAWYQQK PGKAPNLLIY KASTLESGVP 60
SRFSGSGSGT EFTLTISSLQ PDDFATYYCQ QYNTYWTFGQ GTKVEIK 107

M197-G10 HC
EVQLLESGGG LVQPGGSLRL SCAASGFTFS VYAMRWVRQA PGKGLEWVSS IYPSGGKTWY 60
ADSVKGRFTI SRDNSKNTLY LQMNSLRAED TAVYYCAYRR TGIPRRDAFD IWGQGTMVTV 120
SSASTKGPSV FPLAPSSKS 139

M197-G11 LC
QDIQMTQSPS TLSASVGDRV TITCRASQSI SSWLAWYQQK PGKAPNLLIY KASTLESGVP 60
SRFSGSGSGT EFTLTISSLQ PDDFATYYCQ QYNTYWTFGQ GTKVEIK 107

M197-G11 HC
EVQLLESGGG LVQPGGSLRL SCAASGFTFS TYAMVWVRQA PGKGLEWVSS IYPSGGITTY 60
ADSVKGRFTI SRDNSKNTLY LQMNSLRAED TAVYYCAYRR TGIPRRDAFD IWGQGTMVTV 120
SSASTKGPSV FPLAPSSKS 139

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M197-H10              LC
QDIQMTQSPS TLSASVGDRV TITCRASQSI SSWLAWYQQK PGKAPNLLIY KASTLESGVP  60
SRFSGSGSGT EFTLTISSLQ PDDFATYYCQ QYNTYWTFGQ GTKVEIK               107

M197-H10              HC
EVQLLESGGG LVQPGGSLRL SCAASGFTFS SYNMHWVRQA PGKGLEWVSS IVPSGGKTNY  60
ADSVKGRFTI SRDNSKNTLY LQMNSLRAED TAVYYCAYRR TGIPRRDAFD IWGQGTMVTV 120
SSASTKGPSV FPLAPSSKS

M197-H11              LC
QDIQMTQSPS TLSASVGDRV TITCRASQSI SSWLAWYQQK PGKAPNLLIY KASTLESGVP  60
SRFSGSGSGT EFTLTISSLQ PDDFATYYCQ QYNTYWTFGQ GTKVEIK               107

M197-H11              HC
EVQLLESGGG LVQPGGSLRL SCAASGFTFS IYNMIWVRQA PGKGLEWVSS IYPSGGWTTY  60
ADSVKGRFTI SRDNSKNTLY LQMNSLRAED TAVYYCAYRR TGIPRRDAFD IWGQGTMVTV 120
SSASTKGPSV FPLAPSSKS                                              139

M198-A01              LC
QDIQMTQSPS TLSASVGDRV TITCRASQSI SSWLAWYQQK PGKAPNLLIY KASTLESGVP  60
SRFSGSGSGT EFTLTISSLQ PDDFATYYCQ QYNTYWTFGQ GTKVEIK               107

M198-A01              HC
EVQLLESGGG LVQPGGSLRL SCAASGFTFS PYTMIWVRQA PGKLGEWVSS ISSSGGMTPY  60
ADSVKGRFTI SRDNSKNTLY LQMNSLRAED TAVYYCAYRR TGIPRRDAFD IWGQGTMVTV 120
SSASTKGPSV FPLAPSSKS                                              139

M198-A02              LC
QDIQMTQSPS TLSASVGDRV TITCRASQSI SSWLAWYQQK PGKAPNLLIY KASTLESGVP  60
SRFSGSGSGT EFTLTISSLQ PDDFATYYCQ QYNTYWTFGQ GTKVEIK               107

M198-A02              HC
EVQLLESGGG LVQPGGSLRL SCAASGFTFS RYSMIWVRQA PGKGLEWVSS IWSSGGATEY  60
ADSVKGRFTI SRDNSKNTLY LQMNSLRAED TAVYYCAYRR TGIPRRDAFD IWGQGTMVTV 120
SSASTKGPSV FPLAPSSKS                                              139

M198-A06              LC
QDIQMTQSPS TLSASVGDRV TITCRASQSI SSWLAWYQQK PGKAPNLLIY KASTLESGVP  60
SRFSGSGSGT EFTLTISSLQ PDDFATYYCQ QYNTYWTFGQ GTKVEIK               107

M198-A06              HC
EVQLLESGGG LVQPGGSLRL SCAASGFTFS IYSMHWVRQA PGKGLEWVSS IYSSGGPTKY  60
ADSVKGRFTI SRDNSKNTLY LQMNSLRAED TAVYYCAYRR TGIPRRDAFD IWGQGTMVTV 120
SSASTKGPSV FPLAPSSKS                                              139

M198-A07              LC
QDIQMTQSPS TLSASVGDRV TITCRASQSI SSWLAWYQQK PGKAPNLLIY KASTLESGVP  60
SRFSGSGSGT EFTLTISSLQ PDDFATYYCQ QYNTYWTFGQ GTKVEIK               107

M198-A07              HC
EVQLLESGGG LVQPGGSLRL SCAASGFTFS RYQMHWVRQA PGKGLEWVSW ISPSGGITGY  60
ADSVKGRFTI SRDNSKNTLY LQMNSLRAED TAVYYCAYRR TGIPRRDAFD IWGQGTMVTV 120
SSASTKGPSV FPLAPSSKS                                              139

M198-A08              LC
QDIQMTQSPS TLSASVGDRV TITCRASQSI SSWLAWYQQK PGKAPNLLIY KASTLESGVP  60
SRFSGSGSGT EFTLTISSLQ PDDFATYYCQ QYNTYWTFGQ GTKVEIK               107

M198-A08              HC
EVQLLESGGG LVQPGGSLRL SCAASGFTFS WYMMQWVRQA PGKGLEWVSR ISPSGGTTYA  60
DSVKGRFTIS RDNSKNTLYL QMNSLRAEDT AVYYCAYRRT GIPRRDAFDI WGQGTMVTVS 120
SASTKGPSVF PLAPSSKS                                               138

M198-A09              LC
QDIQMTQSPS TLSASVGDRV TITCRASQSI SSWLAWYQQK PGKAPNLLIY KASTLESGVP  60
SRFSGSGSGT EFTLTISSLQ PDDFATYYCQ QYNTYWTFGQ GTKVEIK               107

M198-A09              HC
EVQLLESGGG LVQPGGSLRL SCAASGFTFS IYFMSWVRQA PGKGLEWVSS IRSSGGPTWY  60
ADSVKGRFTI SRDNSKNTLY LQMNSLRAED TAVYYCAYRR TGIPRRDAFD IWGQGTMVTV 120
SSASTKGPSV FPLAPSSKS                                              139

M198-B09              LC
QDIQMTQSPS TLSASVGDRV TITCRASQSI SSWLAWYQQK PGKAPNLLIY KASTLESGVP  60
SRFSGSGSGT EFTLTISSLQ PDDFATYYCQ QYNTYWTFGQ GTKVEIK               107

M198-B09              HC
EVQLLESGGG LVQPGGSLRL SCAASGFTFS VYSMTWVRQA PGKGLEWVSS IGSSGGSTTY  60
ADSVKGRFTI SRDNSKNTLY LQMNSLRAED TAVYYCAYRR TGIPRRDAFD IWGQGTMVTV 120
SSASTKGPSV FPLAPSSKS                                              139
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M198-B12                LC
QDIQMTQSPS TLSASVGDRV TITCRASQSI SSWLAWYQQK PGKAPNLLIY KASTLESGVP 60
SRFSGSGSGT EFTLTISSLQ PDDFATYYCQ QYNTYWTFGQ GTKVEIK             107

M198-B12                HC
EVQLLESGGG LVQPGGSLRL SCAASGFTFS KYSMAWVRQA PGKGLEWVSG IYPSGGRTLY 60
ADSVKGRFTI SRDNSKNTLY LQMNSLRAED TAVYYCAYRR TGIPRRDAFD IWGQGTMVTV 120
SSASTKGPSV FPLAPSSKS                                            139

M198-C03                LC
QDIQMTQSPS TLSASVGDRV TITCRASQSI SSWLAWYQQK PGKAPNLLIY KASTLESGVP 60
SRFSGSGSGT EFTLTISSLQ PDDFATYYCQ QYNTYWTFGQ GTKVEIK             107

M198-C03                HC
EVQLLESGGG LVQPGGSLRL SCAASGFTFS RYSMSWVRQA PGKGLEWVSG ISPSGGETSY 60
ADSVKGRFTI SRDNSKNTLY LQMNSLRAED TAVYYCAYRR TGIPRRDAFD IWGQGTMVTV 120
SSASTKGPSV FPLAPSSKS                                            139

M198-C05                LC
QDIQMTQSPS TLSASVGDRV TITCRASQSI SSWLAWYQQK PGKAPNLLIY KASTLESGVP 60
SRFSGSGSGT EFTLTISSLQ PDDFATYYCQ QYNTYWTFGQ GTKVEIK             107

M198-C05                HC
EVQLLESGGG LVQPGGSLRL SCAASGFTFS RYKMYWVRQA PGKGLEWVSV IGPSGGATFY 60
ADSVKGRFTI SRDNSKNTLY LQMNSLRAED TAVYYCAYRR TGIPRRDAFD IWGQGTMVTV 120
SSASTKGPSV FPLAPSSKS                                            139

M198-C06                LC
QDIQMTQSPS TLSASVGDRV TITCRASQSI SSWLAWYQQK PGKAPNLLIY KASTLESGVP 60
SRFSGSGSGT EFTLTISSLQ PDDFATYYCQ QYNTYWTFGQ GTKVEIK             107

M198-C06                HC
EVQLLESGGG LVQPGGSLRL SCAASGFTFS QYFMHWVRQA PGKGLEWVSY IYPSGGMTEY 60
ADSVKGRFTI SRDNSKNTLY LQMNSLRAED TAVYYCAYRR TGIPRRDAFD IWGQGTMVTV 120
SSASTKGPSV FPLAPSSKS                                            139

M198-C09                LC
QDIQMTQSPS TLSASVGDRV TITCRASQSI SSWLAWYQQK PGKAPNLLIY KASTLESGVP 60
SRFSGSGSGT EFTLTISSLQ PDDFATYYCQ QYNTYWTFGQ GTKVEIK             107

M198-C09                HC
EVQLLESGGG LVQPGGSLRL SCAASGFTFS IYTMYWVRQA PGKGLEWVSS ISPSGGWTYY 60
ADSVKGRFTI SRDNSKNTLY LQMNSLRAED TAVYYCAYRR TGIPRRDAFD IWGQGTMVTV 120
SSASTKGPSV FPLAPSSKS                                            139

M198-C10                LC
QDIQMTQSPS TLSASVGDRV TITCRASQSI SSWLAWYQQK PGKAPNLLIY KASTLESGVP 60
SRFSGSGSGT EFTLTISSLQ PDDFATYYCQ QYNTYWTFGQ GTKVEIK             107

M198-C10                HC
EVQLLESGGG LVQPGGSLRL SCAASGFTFS HYMGMNWVRQ APGKGLEWVS SIVPSGGWTQ 60
YADSVKGRFT ISRDNSKNTL YLQMNSLRAE DTAVYYCAYR RTGIPRRDAF DIWGQGTMVT 120
VSSASTKGPS VFPLAPSSKS                                           140

M198-D12                LC
QDIQMTQSPS TLSASVGDRV TITCRASQSI SSWLAWYQQK PGKAPNLLIY KASTLESGVP 60
SRFSGSGSGT EFTLTISSLQ PDDFATYYCQ QYNTYWTFGQ GTKVEIK             107

M198-D12                HC
EVQLLESGGG LVQPGGSLRL SCAASGFTFS LYVMYWVRQA PGKGLEWVSY IVPSGGPTAY 60
ADSVKGRFTI SRDNSKNTLY LQMNSLRAED TAVYYCAYRR TGIPRRDAFD IWGQGTMVTV 120
SSASTKGPSV FPLAPSSKS                                            139

M198-E02                LC
QDIQMTQSPS TLSASVGDRV TITCRASQSI SSWLAWYQQK PGKAPNLLIY KASTLESGVP 60
SRFSGSGSGT EFTLTISSLQ PDDFATYYCQ QYNTYWTFGQ GTKVEIK             107

M198-E02                HC
EVQLLESGGG LVQPGGSLRL SCAASGFTFS IYSMYWVRQA PGKGLEWVSY IRPSGGNTKY 60
ADSVKGRFTI SRDNSKNTLY LQMNSLRAED TAVYYCAYRR TGIPRRDAFD IWGQGTMVTV 120
SSASTKGPSV FPLAPSSKS                                            139

M198-E09                LC
QDIQMTQSPS TLSASVGDRV TITCRASQSI SSWLAWYQQK PGKAPNLLIY KASTLESGVP 60
SRFSGSGSGT EFTLTISSLQ PDDFATYYCQ QYNTYWTFGQ GTKVEIK             107

M198-E09                HC
EVQLLESGGG LVQPGGSLRL SCAASGFTFS IYDMNWVRQA PGKGLEWVSS IYPSGGRTRY 60
ADSVKGRFTI SRDNSKNTLY LQMNSLRAED TAVYYCAYRR TGIPRRDAFD IWGQGTMVTV 120
SSASTKGPSV FPLAPSSKS                                            139
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M198-E11               LC
QDIQMTQSPS TLSASVGDRV TITCRASQSI SSWLAWYQQK PGKAPNLLIY KASTLESGVP  60
SRFSGSGSGT EFTLTISSLQ PDDFATYYCQ QYNTYWTFGQ GTKVEIK               107

M198-E11               HC
EVQLLESGGG LVQPGGSLRL SCAASGFTFS TYSMYWVRQA PGKGLEWVSS IYPSGGLTWY  60
ADSVKGRFTI SRDNSKNTLY LQMNSLRAED TAVYYCAYRR TGIPRRDAFD IWGQGTMVTV 120
SSASTKGPSV FPLAPSSKS                                              139

M198-F04               LC
QDIQMTQSPS TLSASVGDRV TITCRASQSI SSWLAWYQQK PGKAPNLLIY KASTLESGVP  60
SRFSGSGSGT EFTLTISSLQ PDDFATYYCQ QYNTYWTFGQ GTKVEIK               107

M198-F04               HC
EVQLLESGGG LVQPGGSLRL SCAASGFTFS IYDMWWVRQA PGKGLEWVSS IRPSGGITKY  60
ADSVKGRFTI SRDNSKNTLY LQMNSLRAED TAVYYCAYRR TGIPRRDAFD IWGQGTMVTV 120
SSASTKGPSV FPLAPSSKS                                              139

M198-F08               LC
QDIQMTQSPS TLSASVGDRV TITCRASQSI SSWLAWYQQK PGKAPNLLIY KASTLESGVP  60
SRFSGSGSGT EFTLTISSLQ PDDFATYYCQ QYNTYWTFGQ GTKVEIK               107

M198-F08               HC
EVQLLESGGG LVQPGGSLRL SCAASGFTFS VYSMWWVRQA PGKGLEWVSS ISSSGGMTEY  60
ADSVKGRFTI SRDNSKNTLY LQMNSLRAED TAVYYCAYRR TGIPRRDAFD IWGQGTMVTV 120
SSASTKGPSV FPLAPSSKS                                              139

M198-F09               LC
QDIQMTQSPS TLSASVGDRV TITCRASQSI SSWLAWYQQK PGKAPNLLIY KASTLESGVP  60
SRFSGSGSGT EFTLTISSLQ PDDFATYYCQ QYNTYWTFGQ GTKVEIK               107

M198-F09               HC
EVQLLESGGG LVQPGGSLRL SCAASGFTFS VYNMHWVRQA PGKGLEWVSS IYPSGGMTYY  60
ADSVKGRFTI SRDNSKNTLY LQMNSLRAED TAVYYCAYRR TGIPRRDAFD IWGQGTMVTV 120
SSASTKGPSV FPLAPSSKS                                              139

M198-F12               LC
QDIQMTQSPS TLSASVGDRV TITCRASQSI SSWLAWYQQK PGKAPNLLIY KASTLESGVP  60
SRFSGSGSGT EFTLTISSLQ PDDFATYYCQ QYNTYWTFGQ GTKVEIK               107

M198-F12               HC
EVQLLESGGG LVQPGGSLRL SCAASGFTFS IYTMMWVRQA PGKGLEWVSS IWSSGGQTKY  60
ADSVKGRFTI SRDNSKNTLY LQMNSLRAED TAVYYCAYRR TGIPRRDAFD IWGQGTMVTV 120
SSASTKGPSV FPLAPSSKS                                              139

M198-G03               LC
QDIQMTQSPS TLSASVGDRV TITCRASQSI SSWLAWYQQK PGKAPNLLIY KASTLESGVP  60
SRFSGSGSGT EFTLTISSLQ PDDFATYYCQ QYNTYWTFGQ GTKVEIK               107

M198-G03               HC
EVQLLESGGG LVQPGGSLRL SCAASGFTFS QYTMVWVRQA PGKGLEWVSW IYSSRANYAD  60
SVKGRFTISR DNSKNTLYLQ MNSLRAEDTA VYYCAYRRTG IPRRDAFDIW GQGTMVTVSS 120
ASTKGPSVFP LAPSSKS                                                137

M198-G07               LC
QDIQMTQSPS TLSASVGDRV TITCRASQSI SSWLAWYQQK PGKAPNLLIY KASTLESGVP  60
SRFSGSGSGT EFTLTISSLQ PDDFATYYCQ QYNTYWTFGQ GTKVEIK               107

M198-G07               HC
EVQLLESGGG LVQPGGSLRL SCAASGFTFS IYDMTWVRQA PGKGLEWVSS IYPSGGQTIY  60
ADSVKGRFTI SRDNSKNTLY LQMNSLRAED TAVYYCAYRR TGIPRRDAFD IWGQGTMVTV 120
SSASTKGPSV FPLAPSSKS                                              139

M198-H02               LC
QDIQMTQSPS TLSASVGDRV TITCRASQSI SSWLAWYQQK PGKAPNLLIY KASTLESGVP  60
SRFSGSGSGT EFTLTISSLQ PDDFATYYCQ QYNTYWTFGQ GTKVEIK               107

M198-H02               HC
EVQLLESGGG LVQPGGSLRL SCAASGFTFS PYNMYWVRQA PGKGLEWVSW IVPGGVTKYA  60
DSVKGRFTIS RDNSKNTLYL QMNSLRAEDT AVYYCAYRRT GIPRRDAFDI WGQGTMVTVS 120
SASTKGPSVF PLAPSSKS                                               138

M198-H08               LC
QDIQMTQSPS TLSASVGDRV TITCRASQSI SSWLAWYQQK PGKAPNLLIY KASTLESGVP  60
SRFSGSGSGT EFTLTISSLQ PDDFATYYCQ QYNTYWTFGQ GTKVEIK               107

M198-H08               HC
EVQLLESGGG LVQPGGSLRL SCAASGFTFS IYDMYWVRQA PGKGLEWVSS IGPSGGPTAY  60
ADSVKGRFTI SRDNSKNTLY LQMNSLRAED TAVYYCAYRR TGIPRRDAFD IWGQGTMVTV 120
SSASTKGPSV FPLAPSSKS                                              139
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M198-H09                LC
QDIQMTQSPS TLSASVGDRV TITCRASQSI SSWLAWYQQK PGKAPNLLIY KASTLESGVP  60
SRFSGSGSGT EFTLTISSLQ PDDFATYYCQ QYNTYWTFGQ GTKVEIK               107

M198-H09                HC
EVQLLESGGG LVQPGGSLRL SCAASGFTFS IYLMIWVRQA PGKGLEWVSY IGPSGGPTEY  60
ADSVKGRFTI SRDNSKNTLY LQMNSLRAED TAVYYCAYRR TGIPRRDAFD IWGQGTMVTV 120
SSASTKGPSV FPLAPSSKS                                              139

M199-A08                LC
QDIQMTQSPS TLSASVGDRV TITCRASQSI SSWLAWYQQK PGKAPNLLIY KASTLESGVP  60
SRFSGSGSGT EFTLTISSLQ PDDFATYYCQ QYNTYWTFGQ GTKVEIK               107

M199-A08                HC
EVQLLESGGG LVQPGGSLRL SCAASGFTFS HYIMMWVRQA PGKGLEWVSG IYSSGGITVY  60
ADSVKGRFTI SRDNSKNTLY LQMNSLRAED TAVYYCAYRR IGVPRRDEFD IWGQGTMVTV 120
SSASTKGPSV FPLAPSSKS                                              139

M199-A11                LC
QDIQMTQSPS TLSASVGDRV TITCRASQSI SSWLAWYQQK PGKAPNLLIY KASTLESGVP  60
SRFSGSGSGT EFTLTISSLQ PDDFATYYCQ QYNTYWTFGQ GTKVEIK               107

M199-A11                HC
EVQLLESGGG LVQPGGSLRL SCAASGFTFS HYIMMWVRQA PGKGLEWVSG IYSSGGITVY  60
ADSVKGRFTI SRDNSKNTLY LQMNSLRAED TAVYYCAYRR RGIPRRDAFD IWGQGTMVTV 120
SSASTKGPSV FPLAPSSKS                                              139

M199-B01                LC
QDIQMTQSPS TLSASVGDRV TITCRASQSI SSWLAWYQQK PGKAPNLLIY KASTLESGVP  60
SRFSGSGSGT EFTLTISSLQ PDDFATYYCQ QYNTYWTFGQ GTKVEIK               107

M199-B01                HC
EVQLLESGGG LVQPGGSLRL SCAASGFTFS HYIMMWVRQA PGKGLEWVSG IYSSGGITVY  60
ADSVKGRFTI SRDNSKNTLY LQMNSLRAED TAVYYCAFRR TGIPRRDAFD NWGQGTMVTV 120
SSASTKGPSV FPLAPSSKS                                              139

M200-A10                LC
QDIQMTQSPS TLSASVGDRV TITCRASQSI SSWLAWYQQK PGKAPNLLIY KASTLESGVP  60
SRFSGSGSGT EFTLTISSLQ PDDFATYYCQ QYNTYWTFGQ GTKVEIK               107

M200-A10                HC
EVQLLESGGG LVQPGGSLRL SCAASGFTFS HYIMMWVRQA PGKGLEWVSG IYSSGGITVY  60
ADSVKGRFTI SRDNSKNTLY LQMNSLRAED TAVYYCAYRR TGVPRRDSFD IWGQGTMVTV 120
SSASTKGPSV FPLAPSSKS                                              139

M200-B01                LC
QDIQMTQSPS TLSASVGDRV TITCRASQSI SSWLAWYQQK PGKAPNLLIY KASTLESGVP  60
SRFSGSGSGT EFTLTISSLQ PDDFATYYCQ QYNTYWTFGQ GTKVEIK               107

M200-B01                HC
EVQLLESGGG LVQPGGSLRL SCAASGFTFS HYIMMWVRQA PGKGLEWVSG IYSSGGITVY  60
ADSVKGRFTI SRDNSKNTLY LQMNSLRAED TAVYYCAYRR TGIPRRDAFD SWGQGTMVTV 120
SSASTKGPSV FPLAPSSKS                                              139

M200-D03                LC
QDIQMTQSPS TLSASVGDRV TITCRASQSI SSWLAWYQQK PGKAPNLLIY KASTLESGVP  60
SRFSGSGSGT EFTLTISSLQ PDDFATYYCQ QYNTYWTFGQ GTKVEIK               107

M200-D03                HC
EVQLLESGGG LVQPGGSLRL SCAASGFTFS HYIMMWVRQA PGKGLEWVSG IYSSGGITVY  60
ADSVKGRFTI SRDNSKNTLY LQMNSLRAED TAVYYCAWRR IGVPRRDSFD MWGQGTMVTV 120
SSASTKGPSV FPLAPSSKS                                              139

M200-E11                LC
QDIQMTQSPS TLSASVGDRV TITCRASQSI SSWLAWYQQK PGKAPNLLIY KASTLESGVP  60
SRFSGSGSGT EFTLTISSLQ PDDFATYYCQ QYNTYWTFGQ GTKVEIK               107

M200-E11                HC
EVQLLESGGG LVQPGGSLRL SCAASGFTFS HYIMMWVRQA PGKGLEWVSG IYSSGGITVY  60
ADSVKGRFTI SRDNSKNTLY LQMNSLRAED TAVYYCAYRR TGVPRRDAFD NWGQGTMVTV 120
SSASTKGPSV FPLAPSSKS                                              139

M200-F01                LC
QDIQMTQSPS TLSASVGDRV TITCRASQSI SSWLAWYQQK PGKAPNLLIY KASTLESGVP  60
SRFSGSGSGT EFTLTISSLQ PDDFATYYCQ QYNTYWTFGQ GTKVEIK               107

M200-F01                HC
EVQLLESGGG LVQPGGSLRL SCAASGFTFS HYIMMWVRQA PGKGLEWVSG IYSSGGITVY  60
ADSVKGRFTI SRDNSKNTLY LQMNSLRAED TAVYYCAYRR MGIPRRNAFD IWGQGTMVTV 120
SSASTKGPSV FPLAPSSKS                                              139
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M200-H07                LC
QDIQMTQSPS TLSASVGDRV TITCRASQSI SSWLAWYQQK PGKAPNLLIY KASTLESGVP  60
SRFSGSGSGT EFTLTISSLQ PDDFATYYCQ QYNTYWTFGQ GTKVEIK               107

M200-H07                HC
EVQLLESGGG LVQPGGSLRL SCAASGFTFS HYIMMWVRQA PGKGLEWVSG IYSSGGITVY  60
ADSVKGRFTI SRDNSKNTLY LQMNSLRAED TAVYYCAYRR TGVPRRNAFD NWGQGTMVTV 120
SSASTKGPSV FPLAPSSKS                                              139

M201-A06                LC
QDIQMTQSPS TLSASVGDRV TITCRASQSI SSWLAWYQQK PGKAPNLLIY KASTLESGVP  60
SRFSGSGSGT EFTLTISSLQ PDDFATYYCQ QYNTYWTFGQ GTKVEIK               107

M201-A06                HC
EVQLLESGGG LVQPGGSLRL SCAASGFTFS HYIMMWVRQA PGKGLEWVSG IYSSGGITVY  60
ADSVKGRFTI SRDNSKNTLY LQMNSLRAED TAVYYCAYRR TGIPRRDVFD IWGQGTMVTV 120
SSASTKGPSV FPLAPSSKS                                              139

M201-A07                LC
QDIQMTQSPS TLSASVGDRV TITCRASQSI SSWLAWYQQK PGKAPNLLIY KASTLESGVP  60
SRFSGSGSGT EFTLTISSLQ PDDFATYYCQ QYNTYWTFGQ GTKVEIK               107

M201-A07                HC
EVQLLESGGG LVQPGGSLRL SCAASGFTFS HYIMMWVRQA PGKGLEWVSG IYSSGGITVY  60
ADSVKGRFTI SRDNSKNTLY LQMNSLRAED TAVYYCAYRR TGVPRRDEFD IWGQGTMVTV 120
SSASTKGPSV FPLAPSSKS                                              139

M201-F11                LC
QDIQMTQSPS TLSASVGDRV TITCRASQSI SSWLAWYQQK PGKAPNLLIY KASTLESGVP  60
SRFSGSGSGT EFTLTISSLQ PDDFATYYCQ QYNTYWTFGQ GTKVEIK               107

M201-F11                HC
EVQLLESGGG LVQPGGSLRL SCAASGFTFS HYIMMWVRQA PGKGLEWVSG IYSSGGITVY  60
ADSVKGRFTI SRDNSKNTLY LQMNSLRAED TAVYYCAYRR SGIPRRDAFD IWGQGTMVTV 120
SSASTKGPSV FPLAPSSKS                                              139

M201-H06                LC
QDIQMTQSPS TLSASVGDRV TITCRASQSI SSWLAWYQQK PGKAPNLLIY KASTLESGVP  60
SRFSGSGSGT EFTLTISSLQ PDDFATYYCQ QYNTYWTFGQ GTKVEIK               107

M201-H06                HC
EVQLLESGGG LVQPGGSLRL SCAASGFTFS HYIMMWVRQA PGKGLEWVSG IYSSGGITVY  60
ADSVKGRFTI SRDNSKNTLY LQMNSLRAED TAVYYCAYRR TGVPRRDAFD IWGQGTMVTV 120
SSASTKGPSV FPLAPSSKS                                              139

M201-H08                LC
QDIQMTQSPS TLSASVGDRV TITCRASQSI SSWLAWYQQK PGKAPNLLIY KASTLESGVP  60
SRFSGSGSGT EFTLTISSLQ PDDFATYYCQ QYNTYWTFGQ GTKVEIK               107

M201-H08                HC
EVQLLESGGG LVQPGGSLRL SCAASGFTFS HYIMMWVRQA PGKGLEWVSG IYSSGGITVY  60
ADSVKGRFTI SRDNSKNTLY LQMNSLRAED TAVYYCAYRR TGVPRRDALD NWGQGTMVTV 120
SSASTKGPSV FPLAPSSKS                                              139

M202-A01                LC
QDIQMTQSPS TLSASVGDRV TITCRASQSI SSWLAWYQQK PGKAPNLLIY KASTLESGVP  60
SRFSGSGSGT EFTLTISSLQ PDDFATYYCQ QYNTYWTFGQ GTKVEIK               107

M202-A01                HC
EVQLLESGGG LVQPGGSLRL SCAASGFTFS HYIMMWVRQA PGKGLEWVSG IYSSGGITVY  60
ADSVKGRFTI SRDNSKNTLY LQMNSLRAED TAVYYCAYRR IGVPRRDAFD IWGQGTMVTV 120
SSASTKGPSV FPLAPSSKS                                              139

M202-A04                LC
QDIQMTQSPS TLSASVGDRV TITCRASQSI SSWLAWYQQK PGKAPNLLIY KASTLESGVP  60
SRFSGSGSGT EFTLTISSLQ PDDFATYYCQ QYNTYWTFGQ GTKVEIK               107

M202-A04                HC
EVQLLESGGG LVQPGGSLRL SCAASGFTFS HYIMMWVRQA PGKGLEWVSG IYSSGGITVY  60
ADSVKGRFTI SRDNSKNTLY LQMNSLRAED TAVYYCAYRR KGIPRRDDFD IWGQGTMVTV 120
SSASTKGPSV FPLAPSSKS                                              139

M202-A08                LC
QDIQMTQSPS TLSASVGDRV TITCRASQSI SSWLAWYQQK PGKAPNLLIY KASTLESGVP  60
SRFSGSGSGT EFTLTISSLQ PDDFATYYCQ QYNTYWTFGQ GTKVEIK               107

M202-A08                HC
EVQLLESGGG LVQPGGSLRL SCAASGFTFS HYIMMWVRQA PGKGLEWVSG IYSSGGITVY  60
ADSVKGRFTI SRDNSKNTLY LQMNSLRAED TAVYYCAYRR TGIPRWDAFD IWGQGTMVTV 120
SSASTKGPSV FPLAPSSKS                                              139
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M202-A10             LC
QDIQMTQSPS TLSASVGDRV TITCRASQSI SSWLAWYQQK PGKAPNLLIY KASTLESGVP  60
SRFSGSGSGT EFTLTISSLQ PDDFATYYCQ QYNTYWTFGQ GTKVEIK               107

M202-A10             HC
EVQLLESGGG LVQPGGSLRL SCAASGFTFS HYIMMWVRQA PGKGLEWVSG IYSSGGITVY  60
ADSVKGRFTI SRDNSKNTLY LQMNSLRAED TAVYYCAFRR TGIPRRDSFD IWGQGTMVTV 120
SSASTKGPSV FPLAPSSKS                                              139

M202-A12             LC
QDIQMTQSPS TLSASVGDRV TITCRASQSI SSWLAWYQQK PGKAPNLLIY KASTLESGVP  60
SRFSGSGSGT EFTLTISSLQ PDDFATYYCQ QYNTYWTFGQ GTKVEIK               107

M202-A12             HC
EVQLLESGGG LVQPGGSLRL SCAASGFTFS HYIMMWVRQA PGKGLEWVSG IYSSGGITVY  60
ADSVKGRFTI SRDNSKNTLY LQMNSLRAED TAVYYCAYQR TGVPRRDSFN IWGQGTMVTV 120
SSASTKGPSV FPLAPSSKS                                              139

M202-B03             LC
QDIQMTQSPS TLSASVGDRV TITCRASQSI SSWLAWYQQK PGKAPNLLIY KASTLESGVP  60
SRFSGSGSGT EFTLTISSLQ PDDFATYYCQ QYNTYWTFGQ GTKVEIK               107

M202-B03             HC
EVQLLESGGG LVQPGGSLRL SCAASGFTFS HYIMMWVRQA PGKGLEWVSG IYSSGGITVY  60
ADSVKGRFTI SRDNSKNTLY LQMNSLRAED TAVYYCAYRR TGVPRRDDFD IWGQGTMVTV 120
SSASTKGPSV FPLAPSSKS                                              139

M202-B04             LC
QDIQMTQSPS TLSASVGDRV TITCRASQSI SSWLAWYQQK PGKAPNLLIY KASTLESGVP  60
SRFSGSGSGT EFTLTISSLQ PDDFATYYCQ QYNTYWTFGQ GTKVEIK               107

M202-B04             HC
EVQLLESGGG LVQPGGSLRL SCAASGFTFS HYIMMWVRQA PGKGLEWVSG IYSSGGITVY  60
ADSVKGRFTI SRDNSKNTLY LQMNSLRAED TAVYYCAYRR SGVPRRDDFD IWGQGTMVTV 120
SSASTKGPSV FPLAPSSKS                                              139

M202-C01             LC
QDIQMTQSPS TLSASVGDRV TITCRASQSI SSWLAWYQQK PGKAPNLLIY KASTLESGVP  60
SRFSGSGSGT EFTLTISSLQ PDDFATYYCQ QYNTYWTFGQ GTKVEIK               107

M202-C01             HC
EVQLLESGGG LVQPGGSLRL SCAASGFTFS HYIMMWVRQA PGKGLEWVSG IYSSGGITVY  60
ADSVKGRFTI SRDNSKNTLY LQMNSLRAED TAVYYCAYRR TGVPRWDDFD IWGQGTMVTV 120
SSASTKGPSV FPLAPSSKS                                              139

M202-C02             LC
QDIQMTQSPS TLSASVGDRV TITCRASQSI SSWLAWYQQK PGKAPNLLIY KASTLESGVP  60
SRFSGSGSGT EFTLTISSLQ PDDFATYYCQ QYNTYWTFGQ GTKVEIK               107

M202-C02             HC
EVQLLESGGG LVQPGGSLRL SCAASGFTFS HYIMMWVRQA PGKGLEWVSG IYSSGGITVY  60
ADSVKGRFTI SRDNSKNTLY LQMNSLRAED TAVYYCAYRR PGVPRRDAFD IWGQGTMVTV 120
SSASTKGPSV FPLAPSSKS                                              139

M202-C09             LC
QDIQMTQSPS TLSASVGDRV TITCRASQSI SSWLAWYQQK PGKAPNLLIY KASTLESGVP  60
SRFSGSGSGT EFTLTISSLQ PDDFATYYCQ QYNTYWTFGQ GTKVEIK               107

M202-C09             HC
EVQLLESGGG LVQPGGSLRL SCAASGFTFS HYIMMWVRQA PGKGLEWVSG IYSSGGITVY  60
ADSVKGRFTI SRDNSKNTLY LQMNSLRAED TAVYYCAYRR IGVPRRDDFD IWGQGTMVTV 120
SSASTKGPSV FPLAPSSKS                                              139

M202-D09             LC
QDIQMTQSPS TLSASVGDRV TITCRASQSI SSWLAWYQQK PGKAPNLLIY KASTLESGVP  60
SRFSGSGSGT EFTLTISSLQ PDDFATYYCQ QYNTYWTFGQ GTKVEIK               107

M202-D09             HC
EVQLLESGGG LVQPGGSLRL SCAASGFTFS HYIMMWVRQA PGKGLEWVSG IYSSGGITVY  60
ADSVKGRFTI SRDNSKNTLY LQMNSLRAED TAVYYCAYRR IGVPRRDSFD IWGQGTMVTV 120
SSASTKGPSV FPLAPSSKS                                              139

M202-E03             LC
QDIQMTQSPS TLSASVGDRV TITCRASQSI SSWLAWYQQK PGKAPNLLIY KASTLESGVP  60
SRFSGSGSGT EFTLTISSLQ PDDFATYYCQ QYNTYWTFGQ GTKVEIK               107

M202-E03             HC
EVQLLESGGG LVQPGGSLRL SCAASGFTFS HYIMMWVRQA PGKGLEWVSG IYSSGGITVY  60
ADSVKGRFTI SRDNSKNTLY LQMNSLRAED TAVYYCAYRR TGIPRRDAFE IWGQGTMVTV 120
SSASTKGPSV FPLAPSSKS                                              139
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M202-E06                LC
QDIQMTQSPS TLSASVGDRV TITCRASQSI SSWLAWYQQK PGKAPNLLIY KASTLESGVP  60
SRFSGSGSGT EFTLTISSLQ PDDFATYYCQ QYNTYWTFGQ GTKVEIK               107

M202-E06                HC
EVQLLESGGG LVQPGGSLRL SCAASGFTFS HYIMMWVRQA PGKGLEWVSG IYSSGGITVY  60
ADSVKGRFTI SRDNSKNTLY LQMNSLRAED TAVYYCAYRR RGVPRRDDFD IWGQGTMVTV 120
SSASTKGPSV FPLAPSSKS                                              139

M202-F06                LC
QDIQMTQSPS TLSASVGDRV TITCRASQSI SSWLAWYQQK PGKAPNLLIY KASTLESGVP  60
SRFSGSGSGT EFTLTISSLQ PDDFATYYCQ QYNTYWTFGQ GTKVEIK               107

M202-F06                HC
EVQLLESGGG LVQPGGSLRL SCAASGFTFS HYIMMWVRQA PGKGLEWVSG IYSSGGITVY  60
ADSVKGRFTI SRDNSKNTLY LQMNSLRAED TAVYYCAYRR TGVPRWDAFD IWGQGTMVTV 120
SSASTKGPSV FPLAPSSKS                                              139

M202-G03                LC
QDIQMTQSPS TLSASVGDRV TITCRASQSI SSWLAWYQQK PGKAPNLLIY KASTLESGVP  60
SRFSGSGSGT EFTLTISSLQ PDDFATYYCQ QYNTYWTFGQ GTKVEIK               107

M202-G03                HC
EVQLLESGGG LVQPGGSLRL SCAASGFTFS HYIMMWVRQA PGKGLEWVSG IYSSGGITVY  60
ADSVKGRFTI SRDNSKNTLY LQMNSLRAED TAVYYCAFRR TGVPRRDSFE IWGQGTMVTV 120
SSASTKGPSV FPLAPSSKS                                              139

M202-H03                LC
QDIQMTQSPS TLSASVGDRV TITCRASQSI SSWLAWYQQK PGKAPNLLIY KASTLESGVP  60
SRFSGSGSGT EFTLTISSLQ PDDFATYYCQ QYNTYWTFGQ GTKVEIK               107

M202-H03                HC
EVQLLESGGG LVQPGGSLRL SCAASGFTFS HYIMMWVRQA PGKGLEWVSG IYSSGGITVY  60
ADSVKGRFTI SRDNSKNTLY LQMNSLRAED TAVYYCAFRR TGVPRWDDFD IWGQGTMVTV 120
SSASTKGPSV FPLAPSSKS                                              139

M202-H05                LC
QDIQMTQSPS TLSASVGDRV TITCRASQSI SSWLAWYQQK PGKAPNLLIY KASTLESGVP  60
SRFSGSGSGT EFTLTISSLQ PDDFATYYCQ QYNTYWTFGQ GTKVEIK               107

M202-H05                HC
EVQLLESGGG LVQPGGSLRL SCAASGFTFS HYIMMWVRQA PGKGLEWVSG IYSSGGITVY  60
ADSVKGRFTI SRDNSKNTLY LQMNSLRAED TAVYYCAFRR TGVPRRDVFD IWGQGTMVTV 120
SSASTKGPSV FPLAPSSKS                                              139
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TABLE 9

Sequences of Antibodies Obtained from CDR1/2 and CDR3 Spiking Affinity Maturation Libraries Based on X63-G06

Antibody I.D.	% inhibition at 10 nM	human pKal Ki, app (nM)	LV-CDR1	LC-CDR2	LV-CDR3	HV-CDR1	HV-CDR2	HV-CDR3
M209-F04	97.6	0.09	RTSQFVNSNYLA	GASSRAT	QQSSRTPWT	HYLMT	YISPSGGHTIYADSVKG	VARGIAARSRTSYLDq
M209-C11	96.2	0.14	RTSQFVNSNYLA	GASSRAT	QQSSRTPWT	HYLMT	YISPSGGHTIYADSVKG	VGQGIRGRSRTSYFAq
M206-H08	96.0	0.17	RTSQFVNSNYLA	GASSRAT	QQSSRTPWT	DYMMA	SIVPSGGHTHYADSVKG	VARGIAARSRTSYFDY
M210-C12	95.6	0.16	RTSQFVNSNYLA	GASSRAT	QQSSRTPWT	HYLMT	YISPSGGHTIYADSVKG	VAQGIAARSRTSSVDq
M208-F04	95.4	0.2	RTSQFVNSNYLA	GASSRAT	QQSSRTPWT	HYLMT	YISPSGGHTIYADSVKG	VARGIAARSRTSFFDY
M206-B10	94.7	0.3	RTSQFVNSNYLA	GASSRAT	QQSSRTPWT	qYLMA	SIYPSGGWTKYADSVKG	VARGIAARSRTSYFDY
M208-H02	94.4	0.2	RTSQFVNSNYLA	GASSRAT	QQSSRTPWT	HYLMT	YISPSGGHTIYADSVKG	VARGIASRSRTRYCDY
M210-G04	94.2	0.3	RTSQFVNSNYLA	GASSRAT	QQSSRTPWT	HYLMT	YISPSGGHTIYADSVKG	VATGIVARSRTRYFDq
M210-H06	93.8	0.2	RTSQFVNSNYLA	GASSRAT	QQSSRTPWT	HYLMT	YISPSGGHTIYADSVKG	VARGIAARSRTRYFDY
M208-E10	93.7	0.09	RTSQFVNSNYLA	GASSRAT	QQSSRTPWT	HYLMT	YISPSGGHTIYADSVKG	VAQGISARSRTSYFDY
M209-B09	93.5	0.2	RTSQFVNSNYLA	GASSRAT	QQSSRTPWT	HYLMT	YISPSGGHTIYADSVKG	VAQGIVARSRTSYLHq
M209-C12	93.4		RTSQFVNSNYLA	GASSRAT	QQSSRTPWT	HYLMT	YISPSGGHTIYADSVKG	VGRGIAARSRTSqLDY

TABLE 9-continued

Sequences of Antibodies Obtained from CDR1/2 and CDR3 Spiking Affinity Maturation Libraries Based on X63-G06

Antibody I.D.	% inhibition at 10 nM	human pKal Ki, app (nM)	LV-CDR1	LC-CDR2	LV-CDR3	HV-CDR1	HV-CDR2	HV-CDR3
M208-G03	93.4	0.3	RTSQFVNSNYLA	GASSRAT	QQSSRTPWT	HYLMT	YISPSGGHTIYADSVKG	VARGIAARSRTSYLDY
M206-A06	93.0		RTSQFVNSNYLA	GASSRAT	QQSSRTPWT	NYMMG	SISPSGGLTKYADSVKG	VARGIAARSRTSYFDY
M210-H07	92.8	0.4	RTSQFVNSNYLA	GASSRAT	QQSSRTPWT	HYLMT	YISPSGGHTIYADSVKG	VARGIAARSRTRYFDq
M206-F01	92.6	0.2	RTSQFVNSNYLA	GASSRAT	QQSSRTPWT	GYMMV	RISPSGGPTIYADSVKG	VARGIAARSRTSYFDY
M208-F10	92.5	0.2	RTSQFVNSNYLA	GASSRAT	QQSSRTPWT	HYLMT	YISPSGGHTIYADSVKG	VARGIAARSRTSYFDq
M209-E02	92.4	0.3	RTSQFVNSNYLA	GASSRAT	QQSSRTPWT	HYLMT	YISPSGGHTIYADSVKG	VARGIAARSRTILLDq
M208-C06	91.7	0.4	RTSQFVNSNYLA	GASSRAT	QQSSRTPWT	HYLMT	YISPSGGHTIYADSVKG	VARGIAARSRTSFIDY
M205-D04	91.5	0.4	RTSQFVNSNYLA	GASSRAT	QQSSRTPWT	TYKMq	SISPSGGPTNYADSVKG	VARGIAARSRTSYFDY
M210-G10	91.2	0.4	RTSQFVNSNYLA	GASSRAT	QQSSRTPWT	HYLMT	YISPSGGHTIYADSVKG	VARGIAARSRTSYLDF
M207-A04	90.9		RTSQFVNSNYLA	GASSRAT	QQSSRTPWT	HYLMT	YISPSGGHTIYADSVKG	VARGIAARSRTRSFDY
M210-B02	90.9	0.2	RTSQFVNSNYLA	GASSRAT	QQSSRTPWT	HYLMT	YISPSGGHTIYADSVKG	VARGIAARSRTSYFNq
M208-B01	90.1		RTSQFVNSNYLA	GASSRAT	QQSSRTPWT	HYLMT	YISPSGGHTIYADSVKG	VARGIAARSRTSFFDq
M209-G07	89.8		RTSQFVNSNYLA	GASSRAT	QQSSRTPWT	HYLMT	YISPSGGHTIYADSVKG	VARGIAARSRTSYFDT
M204-A02	89.5		RTSQFVNSNYLA	GASSRAT	QQSSRTPWT	DYMMT	YISPSGGLTSYADSVKG	VARGIAARSRTSYFDY
M206-H01	87.6		RTSQFVNSNYLA	GASSRAT	QQSSRTPWT	EYMMV	RISPSGGTTEYADSVKG	VARGIAARSRTSYFDY
M209-B11	87.3		RTSQFVNSNYLA	GASSRAT	QQSSRTPWT	HYLMT	YISPSGGHTIYADSVKG	VARGIAARSRTRYIDq
M206-F09	86.8		RTSQFVNSNYLA	GASSRAT	QQSSRTPWT	VYMMS	SIVPSGGSTTYADSVKG	VARGIAARSRTSYFDY
M209-C02	86.8		RTSQFVNSNYLA	GASSRAT	QQSSRTPWT	HYLMT	YISPSGGHTIYADSVKG	VARGIAYRRRTSYFDY
M208-G02	86.7		RTSQFVNSNYLA	GASSRAT	QQSSRTPWT	HYLMT	YISPSGGHTIYADSVKG	VARGIADRSRTSYSDY
M205-C11	86.5		RTSQFVNSNYLA	GASSRAT	QQSSRTPWT	QYMMM	RISPSGGSTLYADSVKG	VARGIAARSRTSYFDY
M205-H08	85.9		RTSQFVNSNYLA	GASSRAT	QQSSRTPWT	DYMMM	SIVPSGGHTqYADSVKG	VARGIAARSRTSYFDY
M210-H01	85.5		RTSQFVNSNYLA	GASSRAT	QQSSRTPWT	HYLMT	YISPSGGHTIYADSVKG	VARGIAARSRNSqQDY
M209-D12	85.4		RTSQFVNSNYLA	GASSRAT	QQSSRTPWT	HYLMT	YISPSGGHTIYADSVKG	VARGIAARSRTSYFDq
M209-H09	85.3		RTSQFVNSNYLA	GASSRAT	QQSSRTPWT	HYLMT	YISPSGGHTIYADSVKG	VARGIAARSRTVYFDH
M204-E12	84.1		RTSQFVNSNYLA	GASSRAT	QQSSRTPWT	TYMMq	YIGPSGGKTDYADSVKG	VARGIAARSRTSYFDY
M209-H03	82.6		RTSQFVNSNYLA	GASSRAT	QQSSRTPWT	HYLMT	YISPSGGHTIYADSVKG	VAQGIAARSRTTqFDY
M206-H05	82.5		RTSQFVNSNYLA	GASSRAT	QQSSRTPWT	GYKMq	SISPSGGITMYADSVKG	VARGIAARSRTSYFDY
M209-D03	80.4		RTSQFVNSNYLA	GASSRAT	QQSSRTPWT	HYLMT	YISPSGGHTIYADSVKG	VGRGIAARSRTSFFDq
M205-A02	80.3		RTSQFVNSNYLA	GASSRAT	QQSSRTPWT	TYLMA	GIVSSGGRTLYADSVKG	VARGIAARSRTSYFDY
M208-A10	78.5		RTSQFVNSNYLA	GASSRAT	QQSSRTPWT	HYLMT	YISPSGGHTIYADSVKG	VARGIAARSRTSqFDH
M205-E11	78.2		RTSQFVNSNYLA	GASSRAT	QQSSRTPWT	NYTMG	SISPSGGKTDYADSVKG	VARGIAARSRTSYFDY
M206-E02	77.6		RTSQFVNSNYLA	GASSRAT	QQSSRTPWT	EYMMM	VISPSGGQTHYADSVKG	VARGIAARSRTSYFDY
M205-H01	77.1		RTSQFVNSNYLA	GASSRAT	QQSSRTPWT	NYTMQ	YISPSGGYTGYADSVKG	VARGIAARSRTSYFDY
M207-A02	76.6		RTSQFVNSNYLA	GASSRAT	QQSSRTPWT	HYLMT	YISPSGGHTIYADSVKG	VARGIAARSRTINLDY
M209-H07	76.1		RTSQFVNSNYLA	GASSRAT	QQSSRTPWT	HYLMT	YISPSGGHTIYADSVKG	VARGIAARqRTSYYDY
M209-G01	74.8		RTSQFVNSNYLA	GASSRAT	QQSSRTPWT	HYLMT	YISPSGGHTIYADSVKG	VAgGISGRSRLSYVDY

TABLE 9-continued

Sequences of Antibodies Obtained from CDR1/2 and CDR3 Spiking Affinity Maturation Libraries Based on X63-G06

Antibody I.D.	% inhibition at 10 nM	human pKal Ki, app (nM)	LV-CDR1	LC-CDR2	LV-CDR3	HV-CDR1	HV-CDR2	HV-CDR3
M210-A06	74.8		RTSQFVNSNYLA	GASSRAT	QQSSRTPWT	HYLMT	YISPSGGHTIYADSVKG	VARGIAARSRTSqFDY
M209-D02	74.7		RTSQFVNSNYLA	GASSRAT	QQSSRTPWT	HYLMT	YISPSGGHTIYADSVKG	VARGITARSRTSYFDD
M205-B04	71.1		RTSQFVNSNYLA	GASSRAT	QQSSRTPWT	NYDMI	SISSSGGTTKYADSVKG	VARGIAARSRTSYFDY
M203-A03	69.1		RTSQFVNSNYLA	GASSRAT	QQSSRTPWT	VYMMI	SISPSGGQTTYADSVKG	VARGIAARSRTSYFDY
M209-E03	68.8		RTSQFVNSNYLA	GASSRAT	QQSSRTPWT	HYLMT	YISPSGGHTIYADSVKG	qARGIAARSRTSYFDY
M207-A01	67.2		RTSQFVNSNYLA	GASSRAT	QQSSRTPWT	HYLMT	YISPSGGHTIYADSVKG	VARGISARSRTSCFDY
M206-C03	65.5		RTSQFVNSNYLA	GASSRAT	QQSSRTPWT	qYMMV	SIYSSGGNTPYADSVKG	VARGIAARSRTSYFDY
M207-C05	61.4		RTSQFVNSNYLA	GASSRAT	QQSSRTPWT	HYLMT	YISPSGGHTIYADSVKG	VGRGIAARSRTSYFDK
M205-A12	58.8		RTSQFVNSNYLA	GASSRAT	QQSSRTPWT	QYDMI	YISSSGGFTRYADSVKG	VARGIAARSRTSYFDY
M205-F03	58.6		RTSQFVNSNYLA	GASSRAT	QQSSRTPWT	SqQMV	YISPSGGNTYYADSVKG	VARGIAARSRTSYFDY
M203-A01	51.4		RTSQFVNSNYLA	GASSRAT	QQSSRTPWT	NYLMA	WIVPSGGYTEYADSVKG	VARGIAARSRTSYFDY
M209-B01	47.0		RTSQFVNSNYLA	GASSRAT	QQSSRTPWT	HYLMT	YISPSGGHTIYADSVKG	VARGIVARSRTSNFDq
M208-D12	43.7		RTSQFVNSNYLA	GASSRAT	QQSSRTPWT	HYLMT	YISPSGGHTIYADSVKG	LARGIAARSRTSYqDI
M206-H04	19.0		RTSQFVNSNYLA	GASSRAT	QQSSRTPWT	SYMMV	SISPSGGYTIqADSVKG	VARGIAARSRTSYFDY

Amino Acid Sequences of Light Chain (LC) and Heavy Chain (HC) Variable Domain of pKal Antibodies Obtained from CDR1/2 and CDR3 Spiking Affinity Maturation Libraries Based on X63-G06.

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M203-A01                LC
QDIQMTQSPG TLSLSPGERA TLSCRTSQFV NSNYLAWYQQ TPGQAPRLLI YGASSRATGI   60
PDRFSGTGYG TDFTLTISRL EPEDYGTYYC QQSSRTPWTF GQGTRVEIK             109

M203-A01                HC
EVQLLESGGG LVQPGGSLRL SCAASGFTFS NYLMAWVRQA PGKGLEWVSW IVPSGGYTEY   60
ADSVKGRFTI SRDNSKNTLY LQMNSLRAED TAVYYCARVA RGIAARSRTS YFDYWGQGTL  120
VTVSSASTKG PSVFPLAPSS KS                                          142

M203-A03                LC
QDIQMTQSPG TLSLSPGERA TLSCRTSQFV NSNYLAWYQQ TPGQAPRLLI YGASSRATGI   60
PDRFSGTGYG TDFTLTISRL EPEDYGTYYC QQSSRTPWTF GQGTRVEIK             109

M203-A03                HC
EVQLLESGGG LVQPGGSLRL SCAASGFTFS VYMMIWVRQA PGKGLEWVSS ISPSGGQTTY   60
ADSVKGRFTI SRDNSKNTLY LQMNSLRAED TAVYYCARVA RGIAARSRTS YFDYWGQGTL  120
VTVSSASTKG PSVFPLAPSS KS                                          142

M204-A02                LC
QDIQMTQSPG TLSLSPGERA TLSCRTSQFV NSNYLAWYQQ TPGQAPRLLI YGASSRATGI   60
PDRFSGTGYG TDFTLTISRL EPEDYGTYYC QQSSRTPWTF GQGTRVEIK             109

M204-A02                HC
EVQLLESGGG LVQPGGSLRL SCAASGFTFS DYMMTWVRQA PGKGLqWVSY ISPSGGLTSY   60
ADSVKGRFTI SRDNSKNTLY LQMNSLRAED TAVYYCARVA RGIAARSRTS YFDYWGQGTL  120
VTVSSASTKG PSVFPLAPSS KS                                          142

M204-E12                LC
QDIQMTQSPG TLSLSPGERA TLSCRTSQFV NSNYLAWYQQ TPGQAPRLLI YGASSRATGI   60
PDRFSGTGYG TDFTLTISRL EPEDYGTYYC QQSSRTPWTF GQGTRVEIK             109
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M204-E12                HC
EVQLLESGGG LVQPGGSLRL SCAASGFTFS TYMMqWVRQA PGKGLEWVSY IGPSGGKTDY   60
ADSVKGRFTI SRDNSKNTLY LQMNSLRAED TAVYYCARVA RGIAARSRTS YFDYWGQGTL  120
VTVSSASTKG PSVFPLAPSS KS                                            142

M205-A02                LC
QDIQMTQSPG TLSLSPGERA TLSCRTSQFV NSNYLAWYQQ TPGQAPRLLI YGASSRATGI   60
PDRFSGTGYG TDFTLTISRL EPEDYGTYYC QQSSRTPWTF GQGTRVEIK              109

M205-A02                HC
EVQLLESGGG LVQPGGSLRL SCAASGFTFS TYLMAWVRQA PGKGLEWVSG IVSSGGRTLY   60
ADSVKGRFTI SRDNSKNTLY LQMNSLRAED TAVYYCARVA RGIAARSRTS YFDYWGQGTL  120
VTVSSASTKG PSVFPLAPSS KS                                            142

M205-A12                LC
QDIQMTQSPG TLSLSPGERA TLSCRTSQFV NSNYLAWYQQ TPGQAPRLLI YGASSRATGI   60
PDRFSGTGYG TDFTLTISRL EPEDYGTYYC QQSSRTPWTF GQGTRVEIK              109

M205-A12                HC
EVQLLESGGG LVQPGGSLRL SCAASGFTFS QYDMIWVRQA PGKGLEWVSY ISSSGGFTRY   60
ADSVKGRFTI SRDNSKNTLY LQMNSLRAED TAVYYCARVA RGIAARSRTS YFDYWGQGTL  120
VTVSSASTKG PSVFPLAPSS KS                                            142

M205-B04                LC
QDIQMTQSPG TLSLSPGERA TLSCRTSQFV NSNYLAWYQQ TPGQAPRLLI YGASSRATGI   60
PDRFSGTGYG TDFTLTISRL EPEDYGTYYC QQSSRTPWTF GQGTRVEIK              109

M205-B04                HC
EVQLLESGGG LVQPGGSLRL SCAASGFTFS NYDMIWVRQA PGKGLEWVSS ISSSGGTTKY   60
ADSVKGRFTI SRDNSKNTLY LQMNSLRAED TAVYYCARVA RGIAARSRTS YFDYWGQGTL  120
VTVSSASTKG PSVFPLAPSS KS                                            142

M205-C11                LC
QDIQMTQSPG TLSLSPGERA TLSCRTSQFV NSNYLAWYQQ TPGQAPRLLI YGASSRATGI   60
PDRFSGTGYG TDFTLTISRL EPEDYGTYYC QQSSRTPWTF GQGTRVEIK              109

M205-C11                HC
EVQLLESGGG LVQPGGSLRL SCAASGFTFS QYMMMWVRQA PGKGLEWVSR ISPSGGSTLY   60
ADSVKGRFTI SRDNSKNTLY LQMNSLRAED TAVYYCARVA RGIAARSRTS YFDYWGQGTL  120
VTVSSASTKG PSVFPLAPSS KS                                            142

M205-D04                LC
QDIQMTQSPG TLSLSPGERA TLSCRTSQFV NSNYLAWYQQ TPGQAPRLLI YGASSRATGI   60
PDRFSGTGYG TDFTLTISRL EPEDYGTYYC QQSSRTPWTF GQGTRVEIK              109

M205-D04                HC
EVQLLESGGG LVQPGGSLRL SCAASGFTFS TYKMqWVRQA PGKGLEWVSS ISPSGGPTNY   60
ADSVKGRFTI SRDNSKNTLY LQMNSLRAED TAVYYCARVA RGIAARSRTS YFDYWGQGTL  120
VTVSSASTKG PSVFPLAPSS KS                                            142

M205-E11                LC
QDIQMTQSPG TLSLSPGERA TLSCRTSQFV NSNYLAWYQQ TPGQAPRLLI YGASSRATGI   60
PDRFSGTGYG TDFTLTISRL EPEDYGTYYC QQSSRTPWTF GQGTRVEIK              109

M205-E11                HC
EVQLLESGGG LVQPGGSLRL SCAASGFTFS NYTMGWVRQA PGKGLEWVSS ISPSGGKTDY   60
ADSVKGRFTI SRDNSKNTLY LQMNSLRAED TAVYYCARVA RGIAARSRTS YFDYWGQGTL  120
VTVSSASTKG PSVFPLAPSS KS                                            142

M205-F03                LC
QDIQMTQSPG TLSLSPGERA TLSCRTSQFV NSNYLAWYQQ TPGQAPRLLI YGASSRATGI   60
PDRFSGTGYG TDFTLTISRL EPEDYGTYYC QQSSRTPWTF GQGTRVEIK              109

M205-F03                HC
EVQLLESGGG LVQPGGSLRL SCAASGFTFS SqQMVWVRQA PGKGLEWVSY ISPSGGNTYY   60
ADSVKGRFTI SRDNSKNTLY LQMNSLRAED TAVYYCARVA RGIAARSRTS YFDYWGQGTL  120
VTVSSASTKG PSVFPLAPSS KS                                            142

M205-H01                LC
QDIQMTQSPG TLSLSPGERA TLSCRTSQFV NSNYLAWYQQ TPGQAPRLLI YGASSRATGI   60
PDRFSGTGYG TDFTLTISRL EPEDYGTYYC QQSSRTPWTF GQGTRVEIK              109

M205-H01                HC
EVQLLESGGG LVQPGGSLRL SCAASGFTFS NYTMQWVRQA PGKGLqWVSY ISPSGGYTGY   60
ADSVKGRFTI SRDNSKNTLY LQMNSLRAED TAVYYCARVA RGIAARSRTS YFDYWGQGTL  120
VTVSSASTKG PSVFPLAPSS KS                                            142

M205-H08                LC
QDIQMTQSPG TLSLSPGERA TLSCRTSQF VNSNYLAWYQQ TPGQAPRLLI YGASSRATGI   60
PDRFSGTGYG TDFTLTISRL EPEDYGTYY CQQSSRTPWTF GQGTRVEIK              109
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M205-H08                 HC
EVQLLESGGG LVQPGGSLRL SCAASGFTF SDYMMMWVRQA PGKGLEWVSS IVPSGGHTqY   60
ADSVKGRFTI SRDNSKNTLY LQMNSLRAE DTAVYYCARVA RGIAARSRTS YFDYWGQGTL  120
VTVSSASTKG PSVFPLAPSS KS                                           142

M206-A06                 LC
QDIQMTQSPG TLSLSPGERA TLSCRTSQF VNSNYLAWYQQ TPGQAPRLLI YGASSRATGI   60
PDRFSGTGYG TDFTLTISRL EPEDYGTYY CQQSSRTPWTF GQGTRVEIK              109

M206-A06                 HC
EVQLLESGGG LVQPGGSLRL SCAASGFTF SNYMMGWVRQA PGKGLqWVSS ISPSGGLTKY   60
ADSVKGRFTI SRDNSKNTLY LQMNSLRAE DTAVYYCARVA RGIAARSRTS YFDYWGQGTL  120
VTVSSASTKG PSVFPLAPSS KS                                           142

M206-B10                 LC
QDIQMTQSPG TLSLSPGERA TLSCRTSQF VNSNYLAWYQQ TPGQAPRLLI YGASSRATGI   60
PDRFSGTGYG TDFTLTISRL EPEDYGTYY CQQSSRTPWTF GQGTRVEIK              109

M206-B10                 HC
EVQLLESGGG LVQPGGSLRL SCAASGFTF SqYLMAWVRQA PGKGLEWVSS IYPSGGWTKY   60
ADSVKGRFTI SRDNSKNTLY LQMNSLRAE DTAVYYCARVA RGIAARSRTS YFDYWGQGTL  120
VTVSSASTKG PSVFPLAPSS KS                                           142

M206-C03                 LC
QDIQMTQSPG TLSLSPGERA TLSCRTSQF VNSNYLAWYQQ TPGQAPRLLI YGASSRATGI   60
PDRFSGTGYG TDFTLTISRL EPEDYGTYY CQQSSRTPWTF GQGTRVEIK              109

M206-C03                 HC
EVQLLESGGG LVQPGGSLRL SCAASGFTF SqYMMVWVRQA PGKGLEWVSS IYSSGGNTPY   60
ADSVKGRFTI SRDNSKNTLY LQMNSLRAE DTAVYYCARVA RGIAARSRTS YFDYWGQGTL  120
VTVSSASTKG PSVFPLAPSS KS                                           142

M206-E02                 LC
QDIQMTQSPG TLSLSPGERA TLSCRTSQF VNSNYLAWYQQ TPGQAPRLLI YGASSRATGI   60
PDRFSGTGYG TDFTLTISRL EPEDYGTYY CQQSSRTPWTF GQGTRVEIK              109

M206-E02                 HC
EVQLLESGGG LVQPGGSLRL SCAASGFTF SEYMMMWVRQA PGKGLEWVSV ISPSGGQTHY   60
ADSVKGRFTI SRDNSKNTLY LQMNSLRAE DTAVYYCARVA RGIAARSRTS YFDYWGQGTL  120
VTVSSASTKG PSVFPLAPSS KS                                           142

M206-F01                 LC
QDIQMTQSPG TLSLSPGERA TLSCRTSQF VNSNYLAWYQQ TPGQAPRLLI YGASSRATGI   60
PDRFSGTGYG TDFTLTISRL EPEDYGTYY CQQSSRTPWTF GQGTRVEIK              109

M206-F01                 HC
EVQLLESGGG LVQPGGSLRL SCAASGFTF SGYMMVWVRQA PGKGLEWVSR ISPSGGPTIY   60
ADSVKGRFTI SRDNSKNTLY LQMNSLRAE DTAVYYCARVA RGIAARSRTS YFDYWGQGTL  120
VTVSSASTKG PSVFPLAPSS KS                                           142

M206-F09                 LC
QDIQMTQSPG TLSLSPGERA TLSCRTSQFV NSNYLAWYQQ TPGQAPRLLI YGASSRATGI   60
PDRFSGTGYG TDFTLTISRL EPEDYGTYYC QQSSRTPWTF GQGTRVEIK              109

M206-F09                 HC
EVQLLESGGG LVQPGGSLRL SCAASGFTFS VYMMSWVRQA PGKGLEWVSS IVPSGGSTTY   60
ADSVKGRFTI SRDNSKNTLY LQMNSLRAED TAVYYCARVA RGIAARSRTS YFDYWGQGTL  120
VTVSSASTKG PSVFPLAPSS KS                                           142

M206-H01                 LC
QDIQMTQSPG TLSLSPGERA TLSCRTSQFV NSNYLAWYQQ TPGQAPRLLI YGASSRATGI   60
PDRFSGTGYG TDFTLTISRL EPEDYGTYYC QQSSRTPWTF GQGTRVEIK              109

M206-H01                 HC
EVQLLESGGG LVQPGGSLRL SCAASGFTFS EYMMVWVRQA PGKGLEWVSR ISPSGGTTEY   60
ADSVKGRFTI SRDNSKNTLY LQMNSLRAED TAVYYCARVA RGIAARSRTS YFDYWGQGTL  120
VTVSSASTKG PSVFPLAPSS KS                                           142

M206-H04                 LC
QDIQMTQSPG TLSLSPGERA TLSCRTSQFV NSNYLAWYQQ TPGQAPRLLI YGASSRATGI   60
PDRFSGTGYG TDFTLTISRL EPEDYGTYYC QQSSRTPWTF GQGTRVEIK              109

M206-H04                 HC
EVQLLESGGG LVQPGGSLRL SCAASGFTFS SYMMVWVRQA PGKGLEWVSS ISPSGGYTIq   60
ADSVKGRFTI SRDNSKNTLY LQMNSLRAED TAVYYCARVA RGIAARSRTS YFDYWGQGTL  120
VTVSSASTKG PSVFPLAPSS KS                                           142

M206-H05                 LC
QDIQMTQSPG TLSLSPGERA TLSCRTSQFV NSNYLAWYQQ TPGQAPRLLI YGASSRATGI   60
PDRFSGTGYG TDFTLTISRL EPEDYGTYYC QQSSRTPWTF GQGTRVEIK              109
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M206-H05                 HC
EVQLLESGGG LVQPGGSLRL SCAASGFTFS GYKMqWVRQA PGKGLEWVSS ISPSGGITMY   60
ADSVKGRFTI SRDNSKNTLY LQMNSLRAED TAVYYCARVA RGIAARSRTS YFDYWGQGTL  120
VTVSSASTKG PSVFPLAPSS KS                                          142

M206-H08                 LC
QDIQMTQSPG TLSLSPGERA TLSCRTSQFV NSNYLAWYQQ TPGQAPRLLI YGASSRATGI   60
PDRFSGTGYG TDFTLTISRL EPEDYGTYYC QQSSRTPWTF GQGTRVEIK             109

M206-H08                 HC
EVQLLESGGG LVQPGGSLRL SCAASGFTFS DYMMAWVRQA PGKGLEWVSS IVPSGGHTHY   60
ADSVKGRFTI SRDNSKNTLY LQMNSLRAED TAVYYCARVA RGIAARSRTS YFDYWGQGTL  120
VTVSSASTKG PSVFPLAPSS KS                                          142

M207-A01                 LC
QDIQMTQSPG TLSLSPGERA TLSCRTSQFV NSNYLAWYQQ TPGQAPRLLI YGASSRATGI   60
PDRFSGTGYG TDFTLTISRL EPEDYGTYYC QQSSRTPWTF GQGTRVEIK             109

M207-A01                 HC
EVQLLESGGG LVQPGGSLRL SCAASGFTFS HYLMTWVRQA PGKGLEWVSY ISPSGGHTIY   60
ADSVKGRFTI SRDNSKNTLY LQMNSLRAED TAVYYCARVA RGISARSRTS CFDYWGQGTL  120
VTVSSASTKG PSVFPLAPSS KS                                          142

M207-A02                 LC
QDIQMTQSPG TLSLSPGERA TLSCRTSQFV NSNYLAWYQQ TPGQAPRLLI YGASSRATGI   60
PDRFSGTGYG TDFTLTISRL EPEDYGTYYC QQSSRTPWTF GQGTRVEIK             109

M207-A02                 HC
EVQLLESGGG LVQPGGSLRL SCAASGFTFS HYLMTWVRQA PGKGLEWVSY ISPSGGHTIY   60
ADSVKGRFTI SRDNSKNTLY LQMNSLRAED TALYYCARVA RGIAARSRTI NLDYWGQGTL  120
VTVSSASTKG PSVFPLAPSS KS                                          142

M207-A04                 LC
QDIQMTQSPG TLSLSPGERA TLSCRTSQFV NSNYLAWYQQ TPGQAPRLLI YGASSRATGI   60
PDRFSGTGYG TDFTLTISRL EPEDYGTYYC QQSSRTPWTF GQGTRVEIK             109

M207-A04                 HC
EVQLLESGGG LVQPGGSLRL SCAASGFTFS HYLMTWVRQA PGKGLEWVSY ISPSGGHTIY   60
ADSVKGRFTI SRDNSKNTLY LQMNSLRAED TAVYYCARVA RGIAARSRTR SFDYWGQGTL  120
VTVSSASTKG PSVFPLAPSS KS                                          142

M207-C05                 LC
QDIQMTQSPG TLSLSPGERA TLSCRTSQFV NSNYLAWYQQ TPGQAPRLLI YGASSRATGI   60
PDRFSGTGYG TDFTLTISRL EPEDYGTYYC QQSSRTPWTF GQGTRVEIK             109

R0121-D02 = M0207-C05                 HC
EVQLLESGGG LVQPGGSLRL SCAASGFTFS HYLMTWVRQA PGKGLEWVSY ISPSGGHTIY   60
ADSVKGRFTI SRDNSKNTLY LQMNSLRAED TAVYYCARVG RGIAARSRTS YFDKWGQGTL  120
VTVSSASTKG PSVFPLAPSS KS                                          142

M208-A10                 LC
QDIQMTQSPG TLSLSPGERA TLSCRTSQFV NSNYLAWYQQ TPGQAPRLLI YGASSRATGI   60
PDRFSGTGYG TDFTLTISRL EPEDYGTYYC QQSSRTPWTF GQGTRVEIK             109

M208-A10                 HC
EVQLLESGGG LVQPGGSLRL SCAASGFTFS HYLMTWVRQA PGKGLEWVSY ISPSGGHTIY   60
ADSVKGRFTI SRDNSKNTLY LQMNSLRAED TAVYYCARVA RGIAARSRTS qFDHWGQGTL  120
VTVSSASTKG PSVFPLAPSS KS                                          142

M208-B01                 LC
QDIQMTQSPG TLSLSPGERA TLSCRTSQFV NSNYLAWYQQ TPGQAPRLLI YGASSRATGI   60
PDRFSGTGYG TDFTLTISRL EPEDYGTYYC QQSSRTPWTF GQGTRVEIK             109

M208-B01                 HC
EVQLLESGGG LVQPGGSLRL SCAASGFTFS HYLMTWVRQA PGKGLEWVSY ISPSGGHTIY   60
ADSVKGRFTI SRDNSKNTLY LQMNSLRAED TAVYYCARVA RGIAARSRTS FFDqWGQGTL  120
VTVSSASTKG PSVFPLAPSS KS                                          142

M208-C06                 LC
QDIQMTQSPG TLSLSPGERA TLSCRTSQFV NSNYLAWYQQ TPGQAPRLLI YGASSRATGI   60
PDRFSGTGYG TDFTLTISRL EPEDYGTYYC QQSSRTPWTF GQGTRVEIK             109

M208-C06                 HC
EVQLLESGGG LVQPGGSLRL SCAASGFTFS HYLMTWVRQA PGKGLEWVSY ISPSGGHTIY   60
ADSVKGRFTI SRDNSKNTLY LQMNSLRAED TAVYYCARVA RGIAARSRTS FIDYWGQGTL  120
VTVSSASTKG PSVFPLAPSS KS                                          142

M208-D12                 LC
QDIQMTQSPG TLSLSPGERA TLSCRTSQFV NSNYLAWYQQ TPGQAPRLLI YGASSRATGI   60
PDRFSGTGYG TDFTLTISRL EPEDYGTYYC QQSSRTPWTF GQGTRVEIK             109
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M208-D12                HC
EVQLLESGGG LVQPGGSLRL SCAASGFTFS HYLMTWVRQA PGKGLEWVSY ISPSGGHTIY   60
ADSVKGRFTI SRDNSKNTLY LQMNSLRAED TAVYYCARLA RGIAARSRTS YqDIWGQGTL  120
VTVSSASTKG PSVFPLAPSS KS                                          142

M208-E10                LC
QDIQMTQSPG TLSLSPGERA TLSCRTSQFV NSNYLAWYQQ TPGQAPRLLI YGASSRATGI   60
PDRFSGTGYG TDFTLTISRL EPEDYGTYYC QQSSRTPWTF GQGTRVEIK             109

M208-E10                HC
EVQLLESGGG LVQPGGSLRL SCAASGFTFS HYLMTWVRQA PGKGLEWVSY ISPSGGHTIY   60
ADSVKGRFTI SRDNSKNTLY LQMNSLRAED TAVYYCARVA QGISARSRTS YFDYWGQGTL  120
VTVSSASTKG PSVFPLAPSS KS                                          142

M208-F04                LC
QDIQMTQSPG TLSLSPGERA TLSCRTSQFV NSNYLAWYQQ TPGQAPRLLI YGASSRATGI   60
PDRFSGTGYG TDFTLTISRL EPEDYGTYYC QQSSRTPWTF GQGTRVEIK             109

M208-F04                HC
EVQLLESGGG LVQPGGSLRL SCAASGFTFS HYLMTWVRQA PGKGLEWVSY ISPSGGHTIY   60
ADSVKGRFTI SRDNSKNTLY LQMNSLRAED TAVYYCARVA RGIAARSRTS FFDYWGQGTL  120
VTVSSASTKG PSVFPLAPSS KS                                          142

M208-F10                LC
QDIQMTQSPG TLSLSPGERA TLSCRTSQFV NSNYLAWYQQ TPGQAPRLLI YGASSRATGI   60
PDRFSGTGYG TDFTLTISRL EPEDYGTYYC QQSSRTPWTF GQGTRVEIK             109

M208-F10                HC
EVQLLESGGG LVQPGGSLRL SCAASGFTFS HYLMTWVRQA PGKGLEWVSY ISPSGGHTIY   60
ADSVKGRFTI SRDNSKNTLY LQMNSLRAED TAVYYCARVA RGIAARSRTS YFDqWGQGTL  120
VTVSSASTKG PSVFPLAPSS KS                                          142

M208-G02                LC
QDIQMTQSPG TLSLSPGERA TLSCRTSQFV NSNYLAWYQQ TPGQAPRLLI YGASSRATGI   60
PDRFSGTGYG TDFTLTISRL EPEDYGTYYC QQSSRTPWTF GQGTRVEIK             109

M208-G02                HC
EVQLLESGGG LVQPGGSLRL SCAASGFTFS HYLMTWVRQA PGKGLEWVSY ISPSGGHTIY   60
ADSVKGRFTI SRDNSKNTLY LQMNSLRAED TAVYYCARVA RGIADRSRTS YSDYWGQGTL  120
VTVSSASTKG PSVFPLAPSS KS                                          142

M208-G03                LC
QDIQMTQSPG TLSLSPGERA TLSCRTSQFV NSNYLAWYQQ TPGQAPRLLI YGASSRATGI   60
PDRFSGTGYG TDFTLTISRL EPEDYGTYYC QQSSRTPWTF GQGTRVEIK             109

M208-G03                HC
EVQLLESGGG LVQPGGSLRL SCAASGFTFS HYLMTWVRQA PGKGLEWVSY ISPSGGHTIY   60
ADSVKGRFTI SRDNSKNTLY LQMNSLRAED TAVYYCARVA RGIAARSRTS YLDYWGQGTL  120
VTVSSASTKG PSVFPLAPSS KS                                          142

M208-H02                LC
QDIQMTQSPG TLSLSPGERA TLSCRTSQFV NSNYLAWYQQ TPGQAPRLLI YGASSRATGI   60
PDRFSGTGYG TDFTLTISRL EPEDYGTYYC QQSSRTPWTF GQGTRVEIK             109

M208-H02                HC
EVQLLESGGG LVQPGGSLRL SCAASGFTFS HYLMTWVRQA PGKGLEWVSY ISPSGGHTIY   60
ADSVKGRFTI SRDNSKNTLY LQMNSLRAED TAVYYCARVA RGIASRSRTR YCDYWGQGTL  120
VTVSSASTKG PSVFPLAPSS KS                                          142

M209-B01                LC
QDIQMTQSP GTLSLSPGERA TLSCRTSQFV NSNYLAWYQQ TPGQAPRLLI YGASSRATGI   60
PDRFSGTGY GTDFTLTISRL EPEDYGTYYC QQSSRTPWTF GQGTRVEIK             109

M209-B01                HC
EVQLLESGG GLVQPGGSLRL SCAASGFTFS HYLMTWVRQA PGKGLEWVSY ISPSGGHTIY   60
ADSVKGRFT ISRDNSKNTLY LQMNSLRAED TAVYYCARVA RGIVARSRTS NFDqWGQGTL  120
VTVSSASTK GPSVFPLAPSS KS                                          142

M209-B09                LC
QDIQMTQSP GTLSLSPGERA TLSCRTSQFV NSNYLAWYQQ TPGQAPRLLI YGASSRATGI   60
PDRFSGTGY GTDFTLTISRL EPEDYGTYYC QQSSRTPWTF GQGTRVEIK             109

M209-B09                HC
EVQLLESGG GLVQPGGSLRL SCAASGFTFS HYLMTWVRQA PGKGLEWVSY ISPSGGHTIY   60
ADSVKGRFT ISRDNSKNTLY LQMNSLRAED TAVYYCARVA QGIVARSRTS YLHqWGQGTL  120
VTVSSASTK GPSVFPLAPSS KS                                          142

M209-B11                LC
QDIQMTQSP GTLSLSPGERA TLSCRTSQFV NSNYLAWYQQ TPGQAPRLLI YGASSRATGI   60
PDRFSGTGY GTDFTLTISRL EPEDYGTYYC QQSSRTPWTF GQGTRVEIK             109
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M209-B11                  HC
EVQLLESGG GLVQPGGSLRL SCAASGFTFS HYLMTWVRQA PGKGLEWVSY ISPSGGHTIY   60
ADSVKGRFT ISRDNSKNTLY LQMNSLRAED TAVYYCARVA RGIAARSRTR YIDqWGQGTL  120
VTVSSASTK GPSVFPLAPSS KS                                           142

M209-C02                  LC
QDIQMTQSP GTLSLSPGERA TLSCRTSQFV NSNYLAWYQQ TPGQAPRLLI YGASSRATGI   60
PDRFSGTGY GTDFTLTISRL EPEDYGTYYC QQSSRTPWTF GQGTRVEIK              109

M209-C02                  HC
EVQLLESGG GLVQPGGSLRL SCAASGFTFS HYLMTWVRQA PGKGLEWVSY ISPSGGHTIY   60
ADSVKGRFT ISRDNSKNTLY LQMNSLRAED TAVYYCARVA RGIAYRRRTS YFDYWGQGTL  120
VTVSSASTK GPSVFPLAPSS KS                                           142

M209-C11                  LC
QDIQMTQSP GTLSLSPGERA TLSCRTSQFV NSNYLAWYQQ TPGQAPRLLI YGASSRATGI   60
PDRFSGTGY GTDFTLTISRL EPEDYGTYYC QQSSRTPWTF GQGTRVEIK              109

M209-C11                  HC
EVQLLESGG GLVQPGGSLRL SCAASGFTFS HYLMTWVRQA PGKGLEWVSY ISPSGGHTIY   60
ADSVKGRFT ISRDNSKNTLY LQMNSLRAED TAVYYCAMVG QGIRGRSRTS YFAqWGQGTL  120
VTVSSASTK GPSVFPLAPSS KS                                           142

M209-C12                  LC
QDIQMTQSP GTLSLSPGERA TLSCRTSQFV NSNYLAWYQQ TPGQAPRLLI YGASSRATGI   60
PDRFSGTGY GTDFTLTISRL EPEDYGTYYC QQSSRTPWTF GQGTRVEIK              109

M209-C12                  HC
EVQLLESGGG LVQPGGSLRL SCAASGFTFS HYLMTWVRQA PGKGLEWVSY ISPSGGHTIY   60
ADSVKGRFTI SRDNSKNTLY LQMNSLRAED TAVYYCARVG                        120
VTVSSASTKG PSVFPLAPSS KS                                           142

M0209-D02                 LC
QDIQMTQSPG TLSLSPGERA TLSCRTSQFV NSNYLAWYQQ TPGQAPRLLI YGASSRATGI   60
PDRFSGTGYG TDFTLTISRL EPEDYGTYYC QQSSRTPWTF GQGTRVEIK              109

M209-D02                  HC
EVQLLESGGG LVQPGGSLRL SCAASGFTFS HYLMTWVRQA PGKGLEWVSY ISPSGGHTIY   60
ADSVKGRFTI SRDNSKNTLY LQMNSLRAED TAVYYCARVA RGITARSRTS YFDDWGQGTL  120
VTVSSASTKG PSVFPLAPSS KS                                           142

M209-D03                  LC
QDIQMTQSPG TLSLSPGERA TLSCRTSQFV NSNYLAWYQQ TPGQAPRLLI YGASSRATGI   60
PDRFSGTGYG TDFTLTISRL EPEDYGTYYC QQSSRTPWTF GQGTRVEIK              109

M209-D03                  HC
EVQLLESGGG LVQPGGSLRL SCAASGFTFS HYLMTWVRQA PGKGLEWVSY ISPSGGHTIY   60
ADSVKGRFTI SRDNSKNTLY LQMNSLRAED TAVYYCARVG                        120
VTVSSASTKG PSVFPLAPSS KS                                           142

M209-D12                  LC
QDIQMTQSPG TLSLSPGERA TLSCRTSQFV NSNYLAWYQQ TPGQAPRLLI YGASSRATGI   60
PDRFSGTGYG TDFTLTISRL EPEDYGTYYC QQSSRTPWTF GQGTRVEIK              109

M209-D12                  HC
EVQLLESGGG LVQPGGSLRL SCAASGFTFS HYLMTWVRQA PGKGLEWVSY ISPSGGHTIY   60
ADSVKGRFTI SRDNSKNTLY LQMNSLRAED TAVYYCATVA RGIAARSRTS YFDqWGQGTL  120
VTVSSASTKG PSVFPLAPSS KS                                           142

M209-E02                  LC
QDIQMTQSPG TLSLSPGERA TLSCRTSQFV NSNYLAWYQQ TPGQAPRLLI YGASSRATGI   60
PDRFSGTGYG TDFTLTISRL EPEDYGTYYC QQSSRTPWTF GQGTRVEIK              109

M209-E02                  HC
EVQLLESGGG LVQPGGSLRL SCAASGFTFS HYLMTWVRQA PGKGLEWVSY ISPSGGHTIY   60
ADSVKGRFTI SRDNSKNTLY LQMNSLRAED TAVYYCARVA RGIAARSRTI LLDqWGQGTL  120
VTVSSASTKG PSVFPLAPSS KS                                           142

M209-E03                  LC
QDIQMTQSPG TLSLSPGERA TLSCRTSQFV NSNYLAWYQQ TPGQAPRLLI YGASSRATGI   60
PDRFSGTGYG TDFTLTISRL EPEDYGTYYC QQSSRTPWTF GQGTRVEIK              109

M209-E03                  HC
EVQLLESGGG LVQPGGSLRL SCAASGFTFS HYLMTWVRQA PGKGLEWVSY ISPSGGHTIY   60
ADSVKGRFTI SRDNSKNTLY LQMNSLRAED TAVYYCARqA RGIAARSRTS YFDYWGQGTL  120
VTVSSASTKG PSVFPLAPSS KS                                           142

M209-F04                  LC
QDIQMTQSPG TLSLSPGERA TLSCRTSQFV NSNYLAWYQQ TPGQAPRLLI YGASSRATGI   60
PDRFSGTGYG TDFTLTISRL EPEDYGTYYC QQSSRTPWTF GQGTRVEIK              109
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M209-F04             HC
EVQLLESGGG LVQPGGSLRL SCAASGFTFS HYLMTWVRQA PGKGLEWVSY ISPSGGHTIY   60
ADSVKGRFTI SRDNSKNTLY LQMNSLRAED TAVYYCARVA RGIAARSRTS YLDqWSQGTL  120
VTVSSASTKG PSVFPLAPSS KS                                            142

M209-G01             LC
QDIQMTQSPG TLSLSPGERA TLSCRTSQFV NSNYLAWYQQ TPGQAPRLLI YGASSRATGI   60
PDRFSGTGYG TDFTLTISRL EPEDYGTYYC QQSSRTPWTF GQGTRVEIK               109

M209-G01             HC
EVQLLESGGG LVQPGGSLRL SCAASGFTFS HYLMTWVRQA PGKGLEWVSY ISPSGGHTIY   60
ADSVKGRFTI SRDNSKNTLY LQMNSLRAED TAVYYCARVA qGISGRSRLS YVDYWGQGTL  120
VTVSSASTKG PSVFPLAPSS KS                                            142

M209-G07             LC
QDIQMTQSPG TLSLSPGERA TLSCRTSQFV NSNYLAWYQQ TPGQAPRLLI YGASSRATGI   60
PDRFSGTGYG TDFTLTISRL EPEDYGTYYC QQSSRTPWTF GQGTRVEIK               109

M209-G07             HC
EVQLLESGGG LVQPGGSLRL SCAASGFTFS HYLMTWVRQA PGKGLEWVSY ISPSGGHTIY   60
ADSVKGRFTI SRDNSKNTLY LQMNSLRAED TAVYYCARVA RGIAARSRTS YFDTWGQGTL  120
VTVSSASTKG PSVFPLAPSS KS                                            142

M209-H03             LC
QDIQMTQSPG TLSLSPGERA TLSCRTSQFV NSNYLAWYQQ TPGQAPRLLI YGASSRATGI   60
PDRFSGTGYG TDFTLTISRL EPEDYGTYYC QQSSRTPWTF GQGTRVEIK               109

M209-H03             HC
EVQLLESGGG LVQPGGSLRL SCAASGFTFS HYLMTWVRQA PGKGLEWVSY ISPSGGHTIY   60
ADSVKGRFTI SRDNSKNTLY LQMNSLRAED TAVYYCARVA QGIAARSRTT qFDYWGQGTL  120
VTVSSASTKG PSVFPLAPSS KS                                            142

M209-H07             LC
QDIQMTQSPG TLSLSPGERA TLSCRTSQFV NSNYLAWYQQ TPGQAPRLLI YGASSRATGI   60
PDRFSGTGYG TDFTLTISRL EPEDYGTYYC QQSSRTPWTF GQGTRVEIK               109

M209-H07             HC
EVQLLESGGG LVQPGGSLRL SCAASGFTFS HYLMTWVRQA PGKGLEWVSY ISPSGGHTIY   60
ADSVKGRFTI SRDNSKNTLY LQMNSLRAED TAVYYCARVA RGIAARqRTS YYDYWGQGTL  120
VTVSSASTKG PSVFPLAPSS KS                                            142

M209-H09             LC
QDIQMTQSPG TLSLSPGERA TLSCRTSQFV NSNYLAWYQQ TPGQAPRLLI YGASSRATGI   60
PDRFSGTGYG TDFTLTISRL EPEDYGTYYC QQSSRTPWTF GQGTRVEIK               109

M209-H09             HC
EVQLLESGGG LVQPGGSLRL SCAASGFTFS HYLMTWVRQA PGKGLEWVSY ISPSGGHTIY   60
ADSVKGRFTI SRDNSKNTLY LQMNSLRAED TAVYYCARVA RGIAARSRTV YFDHWGQGTL  120
VTVSSASTKG PSVFPLAPSS KS                                            142

M210-A06             LC
QDIQMTQSPG TLSLSPGERA TLSCRTSQFV NSNYLAWYQQ TPGQAPRLLI YGASSRATGI   60
PDRFSGTGYG TDFTLTISRL EPEDYGTYYC QQSSRTPWTF GQGTRVEIK               109

M210-A06             HC
EVQLLESGGG LVQPGGSLRL SCAASGFTFS HYLMTWVRQA PGKGLEWVSY ISPSGGHTIY   60
ADSVKGRFTI SRDNSKNTLY LQMNSLRAED TAVYYCARVA RGIAARSRTS qFDYWGQGTL  120
VTVSSASTKG PSVFPLAPSS KS                                            142

M210-B02             LC
QDIQMTQSPG TLSLSPGERA TLSCRTSQFV NSNYLAWYQQ TPGQAPRLLI YGASSRATGI   60
PDRFSGTGYG TDFTLTISRL EPEDYGTYYC QQSSRTPWTF GQGTRVEIK               109

M210-B02             HC
EVQLLESGGG LVQPGGSLRL SCAASGFTFS HYLMTWVRQA PGKGLEWVSY ISPSGGHTIY   60
ADSVKGRFTI SRDNSKNTLY LQMNSLRAED TAVYYCASVA RGIAARSRTS YFNqWGQGTL  120
VTVSSASTKG PSVFPLAPSS KS                                            142

M210-C12             LC
QDIQMTQSPG TLSLSPGERA TLSCRTSQFV NSNYLAWYQQ TPGQAPRLLI YGASSRATGI   60
PDRFSGTGYG TDFTLTISRL EPEDYGTYYC QQSSRTPWTF GQGTRVEIK               109

M210-C12             HC
EVQLLESGGG LVQPGGSLRL SCAASGFTFS HYLMTWVRQA PGKGLEWVSY ISPSGGHTIY   60
ADSVKGRFTI SRDNSKNTLY LQMNSLRAED TAVYYCARVA QGIAARSRTS SVDqWGQGTL  120
VTVSSASTKG PSVFPLAPSS KS                                            142
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-continued

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M210-G04              LC
QDIQMTQSPG TLSLSPGERA TLSCRTSQFV NSNYLAWYQQ TPGQAPRLLI YGASSRATGI   60
PDRFSGTGYG TDFTLTISRL EPEDYGTYYC QQSSRTPWTF GQGTRVEIK              109

M210-G04              HC
EVQLLESGGG LVQPGGSLRL SCAASGFTFS HYLMTWVRQA PGKGLEWVSY ISPSGGHTIY   60
ADSVKGRFTI SRDNSKNTLY LQMNSLRAED TAVYYCARVA TGIVARSRTR YFDqWGQGTL  120
VTVSSASTKG PSVFPLAPSS KS                                           142

M210-G10              LC
QDIQMTQSPG TLSLSPGERA TLSCRTSQFV NSNYLAWYQQ TPGQAPRLLI YGASSRATGI   60
PDRFSGTGYG TDFTLTISRL EPEDYGTYYC QQSSRTPWTF GQGTRVEIK              109

M210-G10              HC
EVQLLESGGG LVQPGGSLRL SCAASGFTFS HYLMTWVRQA PGKGLEWVSY ISPSGGHTIY   60
ADSVKGRFTI SRDNSKNTLY LQMNSLRAED TAVYYCARVA RGIAARSRTS YLDFWGQGTL  120
VTVSSASTKG PSVFPLAPSS KS                                           142

M210-H01              LC
QDIQMTQSPG TLSLSPGERA TLSCRTSQFV NSNYLAWYQQ TPGQAPRLLI YGASSRATGI   60
PDRFSGTGYG TDFTLTISRL EPEDYGTYYC QQSSRTPWTF GQGTRVEIK              109

M210-H01              HC
EVQLLESGGG LVQPGGSLRL SCAASGFTFS HYLMTWVRQA PGKGLEWVSY ISPSGGHTIY   60
ADSVKGRFTI SRDNSKNTLY LQMNSLRAED TAVYYCARVA RGIAARSRNS qQDYWGQGTL  120
VTVSSASTKG PSVFPLAPSS KS                                           142

M210-H06              LC
QDIQMTQSPG TLSLSPGERA TLSCRTSQFV NSNYLAWYQQ TPGQAPRLLI YGASSRATGI   60
PDRFSGTGYG TDFTLTISRL EPEDYGTYYC QQSSRTPWTF GQGTRVEIK              109

M210-H06              HC
EVQLLESGGG LVQPGGSLRL SCAASGFTFS HYLMTWVRQA PGKGLEWVSY ISPSGGHTIY   60
ADSVKGRFTI SRDNSKNTLY LQMNSLRAED TAVYYCARVA RGIAARSRTR YFDYWGQGTL  120
VTVSSASTKG PSVFPLAPSS KS                                           142

M210-H07              LC
QDIQMTQSPG TLSLSPGERA TLSCRTSQFV NSNYLAWYQQ TPGQAPRLLI YGASSRATGI   60
PDRFSGTGYG TDFTLTISRL EPEDYGTYYC QQSSRTPWTF GQGTRVEIK              109

M210-H07              HC
EVQLLESGGG LVQPGGSLRL SCAASGFTFS HYLMTWVRQA PGKGLEWVSY ISPSGGHTIY   60
ADSVKGRFTI SRDNSKNTLY LQMNSLRAED TAVYYCARVA RGIAARSRTR YFDqWGQGTL  120
VTVSSASTKG PSVFPLAPSS KS                                           142
```

Example 6

In Vivo Testing of M162-A04 (IgG) and X101-A01

Bradykinin and other bioactive kinins have been previously implicated in carrageenan-induced edema and inflammatory pain (Sharma J. N. et al. (1998) Inflammopharmacology 6, 9-17; Asano M. et al. (1997) Br J Pharmacol 122, 1436-1440; De Campos R. O. et al. (1996) Eur J Pharmacol 316, 277-286). Plasma kallikrein and tissue kallikrein 1 are the two primary kininogenases in mammals (Schmaier A. H. (2008) Int Immunopharmacol 8, 161-165). M162-A04 (M162-A4) (IgG), a specific plasma kallikrein inhibitor, was tested to determine whether it would be effective in carrageenan induced edema. The study design is outlined in Table 10. The route of administration (ROA) for the vehicle (PBS), the antibody, and the positive control (indomethacin) was intra-peritoneal (IP) and was given 30 minutes prior to carrageenan injection (0.1 mL of a 2% carrageenan solution). It is evident from FIG. **2** that antibody doses at 10 mg/kg and above were equally effective in reducing carrageenan-induced edema as the positive control (indomethacin). However, the antibody was not effective in reducing carrageenan-induced thermal hyperalgesia (FIG. **3**). The reason for the dissociation between effectiveness in edema and hyperalgesia are not obvious but may be due to differences in the bioactivity of different kinin metabolites. Lys-desArg9-bradykinin is the most potent agonist of the B1 receptor, which is believed to be primarily involved in pain hypersensitivity (Leeb-Lundberg L. M et al. (2005) Pharmacol Rev 57, 27-77). This kinin metabolite is generated by tissue kallikrein 1, not plasma kallikrein (Schmaier A. H. (2008) Int Immunopharmacol 8, 161-165). This difference in kinin generation and resulting bradykinin receptor activation may account for the unexpected decoupling of edema and hyperalgesia in this model.

Another pKal antibody inhibitor X101-A01 was also tested in the CPE model using the study design shown in Table 10B. The data obtained in FIG. **14** shows that X101-A01 inhibited edema in a dose-dependent manner to an extent that is comparable to that of the positive control (indomethacin).

TABLE 10A

Carrageenan-Induced Paw Edema Study Design to test M162-A04

Group #	Number of Rats	Treatment	Dose (mg/kg)	ROA	Timing relative to carrageenan	Dose Volume (mL/kg)
1	6	Vehicle	N/A	IP	T −30 minutes	20
2	6	559A-M162-A4	3	IP	T −30 minutes	20
3	6	559A-M162-A4	10	IP	T −30 minutes	20
4	6	559A-M162-A4	30	IP	T −30 minutes	20

TABLE 10A-continued

Carrageenan-Induced Paw Edema Study Design to test M162-A04

Group #	Number of Rats	Treatment	Dose (mg/kg)	ROA	Timing relative to carrageenan	Dose Volume (mL/kg)
5	6	Indo-methacin	5	IP	T −30 minutes	20

TABLE 10B

Carrageenan-Induced Paw Edema Study Design to Test X101-A01

Group	Treatment	n	Dose (mg/Kg)	ROA	Timing *	Vol (mL/Kg)
1	Vehicle	10	N/A	IP	−30 min	20
2	X101-A01	10	1	IP	−30 min	20
3	X101-A01	10	3	IP	−30 min	20
4	X101-A01	10	10	IP	−30 min	20
5	X101-A01	10	30	IP	−30 min	20
6	Indomethacin	10	5	IP	−30 min	20

Example 7

Evaluation of Selected Antibody Inhibitors of Plasma Kallikrein

Evaluation of selected optimized antibodies (X81-B01 and X67-D03) is shown in Table 11. Neither antibody has any putative deamidation, isomerization, or oxidation sites.

TABLE 11

Criteria	X81-B01 (IgG)	X67-D03 (IgG)
< nM Ki, app against human pKal	0.2 nM	0.1 nM
< nM Ki, app against rodent pKal	mouse - 11 pM rat - 0.14 nM	mouse - 0.7 nM rat - 0.34 nM
prekallikrein binding	no	no
Specific inhibitor with respect to fXIa, plasmin, and trypsin	yes	yes
Inhibits bradykinin generation	yes	yes
Inhibits pKal in presence of prekallikrein	yes	yes
Competition for binding with aprotinin	yes	yes
Stability in human serum	yes	nd*

*not done; a parental form of this antibody was shown to be stable in serum

Example 8

Epitope Mapping

The region of pKal bound by selected anti-pKal antibodies was investigated using several methods. First, competition assays were used to determine whether the antibodies competed for binding to pKal with known active site-directed inhibitors. Second, antibodies were grouped according to whether they were inhibitors or just binders to pKal. Third, epitopes were investigated using synthetic peptides and peptidic structures based on the sequence and 3-dimensional structure of pKal. These peptidic structures are called "CLIPS" (Chemically Linked Peptides on Scaffolds) and the testing was performed by a fee for service company called Pepscan.

Fourth, antibodies were tested for their ability to inhibit pKal from other species, besides human, where the amino acid sequence of pKal has been determined in order to identify amino acids that may account for the differences in inhibition.

Competition Assays

Using a BIACORE® SPR assay antibodies of interest were tested for competition with a known active site inhibitor of pKal. EPI-KAL2 is potent ($K_{i,app}$=0.1 nM) active site inhibitor of pKal and a Kunitz domain inhibitor based on the first domain of tissue factor pathway inhibitor (Markland (1996) *Iterative optimization of high-affinity protease inhibitors using phage display*. 2. Plasma kallikrein and thrombin. Biochemistry. 35(24):8058-67). Kunitz domains are known active site inhibitors of serine proteases, such as pKal.

The sequence of EPI-KAL2 is:

```
EAMHSFCAFKADDGPCRAAHPRWFFNIFTRQCEEFSYGGCGGNQNRFESL

EECKKMCTRD
```

(amino acids in italics are those that differ from TFPI)

As shown in FIGS. **8**A-**8**B, the antibodies X81-B01 and X67-D03 were competed for binding to pKal in the presence or EPI-KAL2. This result indicates that these antibodies either bind in vicinity of the active site or allosteric changes in the conformation of the pKal-EPI-KAL2 complex prevent antibody binding.

Antibody Binders vs Inhibitors

As shown in Tables 1 and 2, all the unique antibodies discovered by phage display were characterized as being either pKal inhibitors or binders but not inhibitors. Antibodies that inhibit the activity of pKal either bind near the active site and preclude substrate interactions (competitive inhibitors) or that bind away from the active site and induce allosteric changes in the structure of the active site (noncompetitive inhibitors). Antibodies that bind but do not inhibit pKal are unlikely to bind near the active site and may bind the non-catalytic domain (i.e. the apple domain). Table 12 categorizes selected antibodies as being either inhibitors or binders of pKal. Also shown in Table 12 for the listed antibodies, is a demonstration of whether they cross-react with mouse pKal as inhibitors and whether they bind prekallikrein.

TABLE 12

Binding Properties of Selected Anti-pKal Antibodies

Number	Antibody	Binding Category	human Ki, app (nM)	mouse Ki, app (nM)	CLIPS Peptide(s) Identified
1	M6-A06	1) Binder only	no	no	C4
2	M6-D09	2) inhibitor, prekallikrein binder, inhibits mouse and human pKal	5.9	3.9	C1, C5
3	M8-C04	1) Binder only	no	no	

TABLE 12-continued

Binding Properties of Selected Anti-pKal Antibodies

Number	Antibody	Binding Category	human Ki, app (nM)	mouse Ki, app (nM)	CLIPS Peptide(s) Identified
4	M8-G09	1) Binder only	no	no	C1, C4, C6, C7
5	M29-D09	3) inhibitor, does not bind prekallikrein, does not inhibit mouse pKal	0.7	no	C1, C4, C7
6	M35-G04	2) inhibitor, prekallikrein binder, inhibits mouse and human pKal	2.9	8	C1, C4
7	M145-D11	3) inhibitor, does not bind prekallikrein, weak inhibitor of mouse pKal	0.79	800	C1, C4
8	M160-G12	4) inhibitor of both mouse and human pKal, does not bind prekallikrein	5	0.2	C2
9	X55-F01	4) inhibitor of both mouse and human pKal, does not bind prekallikrein	0.4	2	C2, C3
10	X73-H09	4) inhibitor, does not bind prekallikrein, weak inhibitor of human and mouse pKal	20	70	C6
11	X81-B01	4) inhibitor of both mouse and human pKal, does not bind prekallikrein	0.1	0.011	C2, C3, C5, C6
12	A2	5) Negative control, does not bind pKal, binds streptavidin	No binding	No binding	No binding

C1-C7: peptides in pKal identified by CLIPS epitope mapping (see FIGS. 9 and 10A-10C).

C1 corresponds to positions 55-67 of the catalytic domain, C2 to positions 81-94, C3 to positions 101-108, C4 to positions 137-151, C5 to positions 162-178, C6 to positions 186-197, and C7 to positions 214-217.

Epitope Mapping Using CLIPS

The 11 anti-pKal antibodies listed in Table 12, plus one negative control (A2) were tested for binding to 5000 different synthetic CLIPS (Chemically Linked Peptides on Scaffolds) by Pepscan as described below in the CLIP METHODS sections. This analysis led to the identification of peptide regions in pKal that are likely to be a part of the antibody epitope for each of the tested antibodies (FIG. 9).

Clips Methods

The linear and CLIPS peptides were synthesized based on the amino acid sequence of the target protein using standard Fmoc-chemistry and deprotected using trifluoric acid with scavengers. The constrained peptides were synthesized on chemical scaffolds in order to reconstruct conformational epitopes, using Chemically Linked Peptides on Scaffolds (CLIPS) technology (Timmerman et al. (2007). For example, the single looped peptides were synthesized containing a dicysteine, which was cyclized by treating with alpha,alpha'-dibromoxylene and the size of the loop was varied by introducing cysteine residues at variable spacing. If other cysteines besides the newly introduced cysteines were present, they were replaced by alanine. The side-chains of the multiple cysteines in the peptides were coupled to CLIPS templates by reacting onto credit-card format polypropylene PEPSCAN cards (455 peptide formats/card) with a 0.5 mM solution of CLIPS template such as 1,3-bis(bromomethyl)benzene in ammonium bicarbonate (20 mM, pH 7.9)/acetonitrile (1:1 (v/v)). The cards were gently shaken in the solution for 30 to 60 minutes while completely covered in solution. Finally, the cards were washed extensively with excess of H_2O and sonicated in disrupt-buffer containing 1 percent SDS/0.1 percent beta-mercaptoethanol in PBS (pH 7.2) at 70° C. for 30 minutes, followed by sonication in H_2O for another 45 minutes.

The binding of antibody to each peptide were tested in a PEPSCAN-based ELISA. The 455-well credit card format polypropylene cards containing the covalently linked peptides were incubated with primary antibody solution for example consisting of 1 micrograms/mL diluted in blocking solution called SQ (4% horse serum, 5% ovalbumin (w/v) in PBS/1% Tween or diluted in PBS e.g., 20% SQ) overnight. After washing, the peptides were incubated with a 1/1000 dilution of rabbit anti-human antibody peroxidase or goat-anti-human FAB peroxidase for one hour at 25° C. After washing, the peroxidase substrate 2,2'-azino-di-3-ethylbenzthiazoline sulfonate (ABTS) and 2 microliters of 3 percent H_2O_2 were added. After one hour, the color development was measured. The color development was quantified with a charge coupled device (CCD)—camera and an image processing system (as firstly described in Slootstra et al., 1996).

Data Calculation

Raw Data: Optical Density (Arbitrary OD Units)

The raw data are optical values obtained by a CCD-camera. The values mostly range from 0 to 3000, a log scale similar to 1 to 3 of a standard 96-well plate elisa-reader. First the CCD-camera makes a picture of the card before peroxidase coloring and then again a picture after the peroxidase coloring. These two pictures are subtracted from each other which results in the data which is called raw-data. This is copied into the Peplab™ database. Then the values are copied to excel and this file is labeled as raw-data file. One follow-up manipulation is allowed. Sometimes a well contains an air-bubble resulting in a false-positive value, the cards are manually inspected and any values caused by an air-bubble are scored as 0.

Normally assays are not done in replicate (only upon request client request). Replicate tests are usually very similar. In addition, the dataset of thousands of peptides contains many peptides that are similar, thus results are never based on recognition of one peptide but on families of similar peptides. If one or a few peptides do not bind, or exhibit lower binding, in a replicate experiment, a different epitope mapping is not normally attributed.

Timmerman et al. (2007). Functional reconstruction and synthetic mimicry of a conformational epitope using CLIPS™ technology. *J. Mol. Recognit.* 20:283-99

Slootstra et al. (1996). Structural aspects of antibody-antigen interaction revealed through small random peptide libraries, *Molecular Diversity,* 1, 87-96.

Example 9

Analysis of pKal Sequences from Different Species

All available sequence of pKal were obtained from public databases and aligned using ClustalW and regions were highlighted based on solvent accessibility, contact with an active site Kunitz inhibitor, and those peptides identified by CLIPS analysis (FIGS. **10**A-**10**C). Citrated plasma from each of these species was obtained and activated using a commercially available prekallikrein activator (from Enzyme Research Laboratories) according to the instructions of the manufacturer Kallikrein activity was then measured in each of the samples in the presence or absence of X81-B01.

It was found that X81-B01 inhibited pKal from all the species except for pig pKal. Since the CLIPS analysis identified four peptides of pKal that X81-B01 binds to—C2 (positions 81-94), C3 (positions 101-108), C5 (positions 162-178) and C6 (positions 186-197)—differences in the pig pKal sequence that correspond to these peptides were examined to identify potential amino acids changes that account for the lack of inhibition of pig pKal by X81-B01. Peptides C2 and C3 are close in the sequence and are both highly similar in sequence among the different species. However, there is a difference at position 479. All the species except pig, frog, and dog have a serine at position 479. The frog and dog pKal sequence has an alanine and a threonine at position 479, respectively; both of which are considered conservative substitutions for a serine. In contrast, the pig pKal sequence has a leucine at position 479, which is a considerably less conservative substitution for a serine. Peptide C5 in pig pKal is highly similar to the sequences from the other species. However, at position 563, only in the pig pKal is a histidine present (bold in FIG. **10**C). This position in all the other species, except frog, is a tyrosine. In the frog pKal, which is inhibited by X81-B01, this position is a threonine. Peptide C6 in pig pKal is again highly similar to the other sequences. However, only in the pig pKal sequence is position 585 a glutamate (in bold in FIG. **10**C). In all the other species this position is an aspartate. This analysis may indicate potentially critical residues in pKal that interact with X81-B01.

Example 10

In Vitro and In Vivo Assays to Assess Efficacy of a Plasma Kallikrein Binding Protein

Binding to Prekallikrein Vs. Kallikrein:

The advantage of an antibody inhibitor of pKal that does not bind prekallikrein over an antibody that binds prekallikrein can be demonstrated experimentally. For example, an in vitro experiment can be designed to compare the potency of a pKal antibody inhibitor that does not bind prekallikrein (e.g. DX-2922 or DX-2930) with one that binds prekallikrein (e.g. M6-D09) using an activated partial thromboplastin time (APTT) plasma clotting time assay. The APTT assay induces clotting in plasma by the addition of a reagent that specifically activates the contact system component of the intrinsic coagulation pathway, of which the activity of pKal is involved. It is well known in the literature that the inhibition of pKal or that a genetic deficiency in pKal leads to prolonged aPPT (see e.g., Morishita, H., et al., Thromb Res, 1994. 73(3-4): p. 193-204; Wynne Jones, D., et al., Br J Haematol, 2004. 127(2): p. 220-3). An in vitro experiment can be performed to measure the effect of spiking citrated human plasma with different concentrations of either M6-D09 or DX-2922 or DX-2930 on observed clotting times induced using commercially available APTT reagents and a coagulation analyzer (Table 13). It is expected that the observed EC50 for APTT prolongation of M6-D09 will be significantly higher than that of DX-2922 and DX-2930 due to the binding of M6-D09 to the high concentration prekallikrein (~500 nM) in the normal plasma sample. Efficacy of the antibody inhibitor of pKal as demonstrated by prolonging APTT supports the potential therapeutic use of the antibody in treating or preventing cardiovascular disease associated with aberrant clot formation, such as may be observed in atherosclerosis, stroke, vasculitis, aneurism, and patients implanted with ventricular assist devices.

TABLE 13

Study Design to Measure Effect of Antibody Inhibitors of pKal on APTT

Condition	Observed Effect on APPT
No treatment, just plasma	Normal
Prekallikrein depleted plasma control (commercially available)	Maximum prolongation
M6-D09 at low concentration	Normal
M6-D09 at middle concentration	Normal
M6-D09 at high concentration	Prolonged APTT
DX-2922 at low concentration	Prolonged APTT
DX-2922 at middle concentration	Prolonged APTT
DX-2922 at high concentration	Maximum prolongation

Efficacy in a Rat Model of Edema:

An in vivo experiment can also be conducted to demonstrate the increased potency of an antibody inhibitor of pKal that does not bind prekallikrein. The carrageenan-induced paw edema (CPE) model of edema in rats is a common pharmacology model. A group of rats will be treated with escalating doses of M6-D09 and DX-2922 by intraperitoneal (IP) injection prior to injecting carrageenan (e.g. 0.1 mL of a 10% w/v solution) in the paws of the rats (Table 14). It is expected that DX-2922 will be more effective in reducing the observed paw swelling than M6-D09. Efficacy of the antibody supports the therapeutic use of the antibody in various inflammatory diseases that are associated with either swelling (e.g. hereditary angioedema, stroke induced edema, brain edema) or bradykinin mediated inflammation and pain (e.g. rheumatoid arthritis, inflammatory bowel disease).

TABLE 14

Study Design to Observe Effect of Antibody Inhibitors on CPE

Group	Treatment	Example Dose (mg/Kg)	Effect Expected
1	Vehicle	N/A	Maximum swelling
2	Indomethacin	5	Maximum reduction of swelling

TABLE 14-continued

Study Design to Observe Effect of Antibody Inhibitors on CPE

Group	Treatment	Example Dose (mg/Kg)	Effect Expected
	(positive control)		
3	M6-D09	1	No effect on swelling
4	M6-D09	3	No effect on swelling
5	M6-D09	10	Intermediate reduction of swelling
6	DX-2922	1	No effect on swelling
7	DX-2922	3	Intermediate reduction of swelling
8	DX-2922	10	Maximum reduction of swelling

An in vivo experiment was conducted to demonstrate the anti-inflammatory potentcy and efficacy of a plasma kallikrein binding protein, DX-2930 after intraperitoneal and subcutaneous injection in the CPE model of edema in rats.

A group of rats where treated with escalating doses of DX-2930 by intraperitoneal (IP) injection prior to injecting carrageenan (e.g. 0.1 mL of a 1% w/v solution) in the paws of the rats. Paw swelling was measured by plethysmography according fluid displacement using established procedures. Indomethacin, the positive control for this experiment, was administered IP at 5 mg/Kg 30 minutes prior to carrageenan injection. The dose of DX-2930 was varied from 1, 3, 10, and 30 mg/Kg. Injection of 0.1 ml 1% carrageenan into the right hind paw resulted in a maximum 2-fold increase in paw volume four hours after challenge. Pretreatment with 5 mg/kg indomethacin inhibited this response by ~50% for the duration of the study. Intraperitoneal injection of DX-2930 thirty minutes prior to carrageenan challenge resulted in a dose-dependent inhibition of the carrageenan-induced response such that no amelioratory effect was observed at the 1 mg/kg dose, but that at the 30 mg/kg dose effects similar to indomethacin were measured.

DX-2930 was administered SC to rats 24 hours prior to the injection of a 0.1 mL 1% carrageenan solution into the right hind paw. Paw swelling was measured by plethysmography according fluid displacement using established procedures. Indomethacin, the positive control for this experiment, was administered IP at 5 mg/Kg 30 minutes prior to carrageenan injection. The dose of DX-2930 was varied from 1, 3, 10, and 30 mg/Kg. In contrast, subcutaneous injection of DX-2930 twenty-four hours prior to carrageenan challenge not only dose-dependently inhibited the carrageenan response, this treatment regimen yielded a significant improvement over indomethacin, delayed the development of the carrageenan-induced edema and significantly inhibited the carrageenan response at all doses throughout the time course of the study.

Measuring Half-Life:

The pharmacokinetic properties of DX-2922 and DX-2930 were determined in rats and the pharmacokinetic properties of DX-2930 was determined in cynomolgus monkeys. Serum was collected at the times indicated below. The concentration of DX-2922 and DX-2930 was determined by ELISA and plotted versus time in order to obtain pharmacokinetic parameters (clearance, half life, volume of distribution, etc).

Pharmacokinetics of DX-2922 and DX-2930 Following Single Intravenous, Subcutaneous or Intraperitoneal Administration in Rats

The objective of this study was to evaluate the pharmacokinetics of DX-2922 and DX-2930, antibody inhibitors of plasma kallikrein, following a single intravenous (IV), subcutaneous (SC), or intraperitoneal (IP) injection to male Sprague-Dawley rats.

Forty two male Sprague-Dawley rats were assigned to 7 dose groups each consisting of 6 animals. All animals were dosed on Day 0. Groups 1 through 3 received a single IV injection of 1 mg/kg, 10 mg/kg, or 20 mg/kg DX-2922, respectively. Groups 4 and 5 received a single SC or IP injection of 20 mg/kg DX-2922, respectively. Groups 6 and 7 received a single IV or SC injection of 20 mg/kg DX-2930, respectively. On Day 0, blood was collected from 3 animals/group (Cohort 1) approximately 5 minutes and 4 hours post-dose. The 3 remaining animals/group (Cohort 2), were bled approximately 1 hour post-dose. All animals from each group were bled on Days 1, 2, 4, 7, 10, 14, 18, and 21. Serum samples were analyzed using a qualified ELISA method. Pharmacokinetic parameters were calculated using WinNonlin Professional Version 5.3 (Pharsight Inc., Cary, N.C.). All data were analyzed noncompartmentally. The study design is summarized in Table 15.

TABLE 15

Study Design

Group	Test Article	Dose Level (mg/kg/day)	Route	Dose Concentration (mg/mL)	Dose Volume (mL/kg)	# Animals
1	DX-2922	1	V	0.5	2	6
2	DX-2922	10	V	5	2	6
3	DX-2922	20	V	10	2	6
4	DX-2922	20	C	10	2	6
5	DX-2922	20	P	10	2	6
6	DX-2930	20	V	10	2	6
7	DX-2930	20	C	10	2	6

The pharmacokinetic parameter estimates are summarized in Table 16.

TABLE 16

Summary of Mean Pharmacokinetic Parameters

Group	C_{max} (μg/mL)	AUC_{last} (hr * μg/mL)	CL (mL/hr/Kg)	Vss (mL/Kg)	t½ (hr)	F (%)
1	18.2	459.4	1.79	461.6	267.5	n/a
2	204.5	5178.5	1.72	314.2	204.6	n/a
3	384.4	9683.0	1.91	279.6	156.4	n/a
4	14.3	1912.4	10.23*	n/a	115.4	20%
5	0.12	26.12	629.93*	n/a	200.53	0.3%
6	414.6	39556.6	0.41	120.0	219.8	n/a
7	91.8	20421.3	0.97*	n/a	57.7	52%

n/a: not applicable
*CL/F

DX-2922 serum concentrations were detected from 5 minutes post-dose to 504 hours (21 Days) post-dose in all dose groups. Mean C_{max} and AUC_{last} values following IV dosing were proportional to dose and increased in a linear fashion with increasing dose. IV clearance was rapid and independent of dose with mean values ranging from 1.72 mL/hr/Kg to 1.91 mL/hr/Kg across dose groups. Mean elimination half-life values decreased with increasing dose and ranged from 268 hours in the 1 mg/kg IV dose group to 156 hours in the 20 mg/kg dose group. Volume of distribution was greater than serum volume suggesting extravascular distribution. Following SC and IP dosing, the mean elimination half-life was 115 hours and 201 hours, respectively. The relative bioavailability when administered by the SC and IP routes were approximately 20% and 0.3%, respectively.

DX-2930 serum concentrations were detected from 5 minutes post-dose to 504 hours (21 Days) post-dose in all dose groups. Following IV dosing, mean C_{max} and AUC_{last} values were 415 μg/mL and 39557 μg/mL*hr, respectively. Mean clearance and elimination half-life values were 0.41 mL/hr/kg and 220 hours, respectively. Volume of distribution was consistent with serum volume suggesting limited extravascular distribution. The relative bioavailability when administered by the SC route was approximately 52%.

The mean serum concentration data for DX-2930 are shown graphically in FIG. **17** and FIG. **18**.

Example 11

Epitope Mapping Using Amino Acid Mutations of pKal

Based on the epitope mapping studies described herein in Example 8, we inspected the published 3 dimensional model in the RCSB Protein Data Bank (available on the world wide web at rcsb.org; pdb code 2ANY) and identified a collection of sets of amino acids in surface accessible loops near the enzyme active site that we reasoned could interact with the antibody binding resulting in enzyme inhibition. These amino acids were substituted for alanine and the catalytic domain of each of the mutant was expressed in *Pichia pastoris* with a His tag fusion and purified by IMAC. Four different mutant pKal mutants were synthesized and tested:

Mutant 1: Amino acids S478, N481, S525, and K526 of the human kallikrein sequence (Accession No. NP_00883.2) were mutated to alanine. These amino acids were determined to be involved in substrate recognition (S3 subsite).

Mutant 2: Amino acid residues R551, Q553, Y555, T558 and R560 of the human kallikrein sequence (Accession No. NP_00883.2) were mutated to alanine. It was determined that these residues are involved in the active site substrate recognition (S1' subsite).

Mutant 3: Amino acids D572, K575, and D577 of the human kallikrein sequence (Accession No. NP_00883.2) were mutated to alanine. These amino acid residues are involved in substrate recognition (S1' subsite)

Mutant 4: Amino acids N395, 5397 and 5398 of the human kallikrein sequence (Accession No. NP_00883.2) were mutated to alanine. These residues are distal from the active site of plasma kallikrein.

Three of the 4 mutants (Mutant 1, 2, and 4) have similar activity to that of the wildtype catalytic domain of pKal. The amino acid substitutions in Mutant 3 yielded an inactive protein that was not recognized in SPR (Biacore) binding assays by any of the tested anti-pKal antibodies.

The antibodies tested for inhibition of mutants 1, 2 and 4 are shown herein in Table 17. Based on the measured $K_{i,app}$ values for the antibodies in Group 1 (i.e., antibodies that inhibit human and mouse pKal but do not bind prekallikrein) it is evident that this group of antibodies binds an epitope on pKal that contains the amino acids that were mutated in Mutant 2 but were not dependent on residues mutated in Mutants 1 or 4. In addition, the interaction of plasma kallikrein binding proteins X81-B01/X101-A01/DX-2922 and affinity matured derivative X115-B07 to kallikrein is adversely affected by the substitutions in Mutant 1. For an example of the differences in the ability of the antibodies to bind prekallikrein see e.g., FIGS. **11**A and **11**B, which compares prekallikrein the binding of DX-2922 (Group 1) to that of M6-D09 (Group 3).

The antibodies in Group 2 (i.e., those that inhibit human pKal not mouse pkal and do not bind prekallikrein) were not significantly affected by the mutated amino acids indicating that they make contact with alternate amino acids. The Group 2 antibodies are likely to bind near the active site, as they were unable to bind pKal complexed with a Kunitz domain (EPI-KAL2), which are known to bind at the active site of a serine protease. Furthermore, one of the antibodies in Group 2 (M145-D11) is similar to those in Group 1 in that it is unable to bind pKal in a Biacore assay that is inactivated with the suicide inhibitor AEBSF (4-(2-Aminoethyl)benzenesulfonyl fluoride hydrochloride), which is a small molecule covalent inhibitor of trypsin-like serine proteases (FIG. **12**). However, the other antibody (M29-D09) assigned to Group 2 was able to bind AEBSF inactivated pKal, indicating that it may bind a different epitope than M145-D11 despite sharing similar binding properties.

The antibodies in Group 3 inhibited human and mouse pKal but bound prekallikrein. One of these antibodies, M6-D09, was unable to bind pKal inactivated by either EPI-KAL2 or AEBSF, indicating that this group of pKal inhibitors interacts with alternative amino acids near the active site. The $K_{i,app}$ for M6-D09 increased approximately 5-fold towards Mutant 2 (i.e., decreased potency of M6-D09).

Example 12

Affinity Maturation

In addition to the affinity maturation described herein in Examples 4 and 5, which involved optimization of the light chain we attempted to further optimize affinity with libraries that vary amino acids in the CDR1, CDR2, and CDR3 regions of the variable heavy chain of two different parental anti-pKal antibodies. Both of the antibodies selectedfor further optimization (X63-G06 and M162-A04) exhibit desirable properties for further development as a therapeutic antibody inhibitor of plasma kallikrein; properties which include: a) complete inhibition of human and rodent plasma kallikrein and b) no binding to prekallikrein. In some embodiments, complete inhibition of human pKal is essential to block the activity of plasma kallikrein in disease uses. Inhibition of rodent pKal facilitates preclinical development including toxicity assessment. The lack of binding to prekallikrein is a highly desirable property for an antibody inhibitor of pKal to maximize the bioavailability of the antibody therapeutic towards active pKal target and to potentially reduce the dose required for efficacy.

Affinity maturation was performed using 4 different phage display libraries. For each parental antibody (e.g., I62-A04), a library was constructed that contained varied amino acid positions in both the CDR1 and the CDR2 of the heavy chain. An additional library was constructed for each of the two parental antibodies wherein positions in the CDR3 of the heavy chain were varied. Each of these 4 phage display libraries were selected (panned) with decreasing amounts of active pKal in each subsequent round in order to obtain high affinity antibodies. To minimize the appearance of prekallikrein binding in the selected antibody output libraries were initially depleted against immobilized prekallikrein. After screening as Fab fragments, we discovered the affinity matured antibodies shown in Table 16 (i.e. the antibodies with the identification number starting with “X115”).

Four discovered antibodies (X115-B07, X115-D05, X115-E09, and X115-H06) are derived from the DX-2922 parental antibody (also known as X63-G06 as a Fab fragment, X81-B01 as an IgG produced in 293T cells, or X101-A01 as an IgG produced in CHO cells) were found to be potent pKal inhibitors. For comparison the amino acid sequence of DX-2922 is shown. It is evident that three of the affinity matured antibodies (X115-B07, X115-E09, and X115-H06) contain mutations in Hv-CDR3; whereas X115-D05 has a different Hv-CDR1/CDR2. Four other discovered antibodies (X115-F02, X115-A03, X115-D01, and X115-G04) are derived from the M162-A04 parental antibody. All 8 affinity matured antibodies do not bind prekallikrein.

TABLE 17

Summary of Affinity Matured Anti-pKal Antibodies Inhibition Constants (Ki, app) on Wild Type pKal Catalytic Domain and Mutants 1, 2, and 4[a].

Isolate	WT cat. Domain Ki, app (nM)	Mutant 1 Ki, app (nM)	Mutant 2 Ki, app (nM)	Mutant 4 Ki, app (nM)	Competes with AEBSF	Competes with epi-kal2	Characteristics
DX-2922	0.22	14	20	0.25	y	y	inhibits human and mouse pKal; does not bind pre-kallikrein
559A-X115-B07 (aff mat; X101-A01 parent)	0.13	4.7	47	0.14	y	nd	inhibits human and mouse pKal; does not bind pre-kallikrein
559A-X115-D05 (aff mat; X101-A01 parent)	nd	nd	nd	nd	y	nd	inhibits human and mouse pKal; does not bind pre-kallikrein
559A-X115-E09 (aff mat; X101-A01 parent)	nd	nd	nd	nd	y	nd	inhibits human and mouse pKal; does not bind pre-kallikrein
559A-X115-H06 (aff mat; X101-A01 parent)	nd	nd	nd	nd	y	nd	inhibits human and mouse pKal; does not bind pre-kallikrein
559A-X115-A03 (aff mat; M162-A04 parent)	0.16	0.23	3.7	0.13	y	nd	inhibits human and mouse pKal; does not bind pre-kallikrein
559A-X115-D01 (aff mat; M162-A04 parent)	0.18	0.26	2.5	0.12	y	nd	inhibits human and mouse pKal; does not bind pre-kallikrein
559A-X115-F02 (aff mat; M162-A04 parent)	0.09	0.14	5.9	0.1	y	y	inhibits human and mouse pKal; does not bind pre-kallikrein
559A-X115-G04 (aff mat; M162-A04 parent)	0.3	0.4	2.2	0.3	y	y	inhibits human and mouse pKal; does not bind pre-kallikrein
559A-M29-D09 (sFab))	0.24	0.27	0.34	0.39	nd	y	inhibits human and mouse pKal; does not inhibit mouse pKal; does not bind pre-kallikrein
559A-M145-D11 (sFab)	0.16	0.23	0.1	0.21	y	y	inhibits human and mouse pKal; weakly inhibits mouse pKal; does not bind pre-kallikrein
559A-M06-D09	2.5	3.4	13.5	2.9	y	y y	inhibits human and mouse pKal; binds pre-kallikrein
559A-M35-G04	0.8	0.09	1.1	0.8	nd	nd	inhibits human and mouse pKal; binds pre-kallikrein

[a]Antibodies were obtained from HV-CDR1/2 and HV-CDR3 affinity maturation, purified and tested for inhibition of either wild type pKal catalytic domain (Note, the antibodies inhibited full length wild type pKal approximately equal to that of the wild type catalytic domain).

TABLE 18

Isolate	LV-CDR1	LV-CDR2	LV-CDR3	HV-CDR1	HV-CDR2	HV-CDR3	Full length pKal Ki, app (nM)
DX-2922	RTSQFVNSNYLA	GASSRAT	QQSSRTPWT	HYLMT	YISPSGGHTIYADSVKG	VARGIAARSRTSYFDY	0.2
X115-B07	RTSQFVNSNYLA	GASSRAT	QQSSRTPWT	HYLMT	YISPSGGHTIYADSVKG	V**GQ**GI**RG**RSRTSYF**AQ**	0.33
X115-D05	RTSQFVNSNYLA	GASSRAT	QQSSRTPWT	**DYMMA**	**SIVPSGGHTHYADSVKG**	VARGIAARSRTSYFDY	0.25
X115-E09	RTSQFVNSNYLA	GASSRAT	QQSSRTPWT	HYLMT	YISPSGGHTIYADSVKG	VA**Q**GIAARSRTS**SVDQ**	0.34
X115-H06	RTSQFVNSNYLA	GASSRAT	QQSSRTPWT	HYLMT	YISPSGGHTIYADSVKG	VA**Q**GI**S**ARSRTSYFDY	0.35
M162-A04	RASQSISSWLA	KASTLES	QQYNTYWT	HYIMM	GIYSSGGITVYADSVKG	RRTGIPRRDAFDI	
X115-A03	RASQSISSWLA	KASTLES	QQYNTYWT	HYIMM	GIYSSGGITVYADSVKG	RR**I**G**V**PRRD**S**FD**M**	0.16
X115-D01	RASQSISSWLA	KASTLES	QQYNTYWT	**IYSMH**	**SIYPSRGMTWYADSVKG**	RRTGIPRRDAFDI	0.18
X115-F02	RASQSISSWLA	KASTLES	QQYNTYWT	HYIMM	GIYSSGGITVYADSVKG	RR**I**G**V**PRRD**E**FDI	0.089
X115-G04	RASQSISSWLA	KASTLES	QQYNTYWT	HYIMM	GIYSSGGITVYADSVKG	RRTG**V**PRRD**E**FDI	0.6
M29-D09	SGNKLGDKYVA	QDTKRPS	QAWDSSIVI	WYTMV	YIYPSGGATFYADSVKG	GSYDYIWGFYSDH	0.7
M145-D11	SGDKLGDKYTS	QDIKRPS	QAWDSPNARV	HYRMS	SIYPSGGRTVYADSVKG	DKFEWRLLFRGIGNDAFDI	0.79
M06-D09	RASQSIRNYLN	AASTLQS	QQLSGYPHT	FYYMV	VIYPSGGITVYADSVKG	DKWAVMPPYYYYAMDV	5.9
M35-G04	RASQSVSSYLA	DASNRAT	QQRSNWPRGFT	YYHMS	VISPSGGSTKYADSVKG	GGSSDYAWGSYRRPYYFDY	2.9

TABLE 18-continued

Isolate	WT cat. Domian Ki, app (nm)	Mutant 1 Ki, app (nm)	Mutant 2 Ki, app (nm)	Mutant 4 Ki, app (nm)	competes with AEBSF	competes with epi-kal2	Group
DX-2922	0.22	14	20	0.25	V	y	1
X115-B07		4.7	47	0.14	y	nd	1
X115-D05	nd	nd	nd	nd	y	nd	1
X115-E09	nd	nd	nd	nd	y	nd	1
X115-H06	nd	nd	nd	nd	y	nd	1
M162-A04	nd	nd	nd	nd	y	y	1
X115-A03	0.16	0.23	3.7	0.13	y	nd	1
X115-D01	0.18	0.26	2.5	0.12	y	nd	1
X115-F02	0.09	0.14	5.9	0.1	y	y	1
X115-G04	0.3	0.4	2.2	0.3	y	y	1
M29-D09	0.24	0.27	0.34	0.39	n	y	2
M145-D11	0.16	0.23	0.1	0.21	y	y	2
M06-D09	2.5	3.4	13.5	2.9	y	y	3
M35-G04	0.8	0.09	1.1	0.8	nd	nd	3

Equilibrium $K_{i,app}$ Measurements.

Apparent Inhibition constants ($K_{i,app}$ values) were measured by pre-incubating enzyme and inhibitor solutions prior to initiating the reactions with substrate. Enzyme and inhibitor were pre-incubated for 2 hours at 30° C. in a 96-well plate by adding 10 μL of a 10× enzyme solution and 10 μL of 10× inhibitor solutions to 70 μL of reaction buffer. Reactions were initiated by the addition of 10 μL of a 10× concentrated stock of substrate, and were monitored at 30° C. in a fluorescence plate reader with the excitation and emission wavelengths set at 360 nm/460 nm, respectively. Kinetic data were acquired by the increase in fluorescence, and initial rates for each condition were plotted against the total inhibitor concentration. The data was fit to the following equation for tight binding inhibitors:

$$A = A_0 - A_{inh}\left(\frac{(K_{i,app} + Inh + E) - \sqrt{(K_{i,app} + Inh + E)^2 - 4 \cdot Inh \cdot E}}{2 \cdot E}\right) \quad \text{Eqn. 1}$$

Where A=initial rate observed at each inhibitor concentration; A_o=initial rate observed in the absence of inhibitor; A_{inh}=initial rate observed for the enzyme inhibitor complex; Inh=concentration of inhibitor; E=total enzyme concentration (treated as a floated parameter); and $K_{i,app}$=apparent equilibrium inhibition constant.

Groups of Antibody Inhibitors.

Antibodies in Group 1 inhibit human and mouse pKal but do not bind prekallikrein. Antibodies in Group 2 inhibit human but not mouse pKal and do not bind prekallikrein. Antibodies in Group 3 inhibit human and mouse pKal but bind prekallikrein.

Biacore Competition Analysis with an Exemplary Kallikrein Antibody, Epi-Kal2.

Epi-Kal2 is an antibody inhibitor of kallikrein that acts by binding to the active site of kallikrein (for sequence see Example 8). The Biacore competition analysis is used herein as an assay to determine whether a test kallikrein antibody binds to the same site as epi-Kal2 and is assessed by measuring the competition (e.g., displacement) between epi-Kal2 and the test antibody for binding to the active site.

Goat anti-human Fc fragment specific IgG or anti-human Fab IgG was immobilized by amine coupling on a CM5 sensor chip at immobilization densities of approximately 5000 RU. Anti-pKal antibodies or sFabs were captured on their respective surfaces by injecting a 50 nM solution of IgG/sFab for 1-2 minutes at 5 at μl/min Human pKal (100 nM) or human pKal-ep-kal2 complex (100 nM hpKal that had been pre-incubated with 1 μM epi-kal2 for 1 hour at room temperature) were injected over the captured IgGs or sFabs for 5 minutes at 20-50 μl/min followed by a 5-10 minute dissociation phase. Binding responses were recorded at the end of the association phase. Anti-pKal IgGs or sFabs were considered to compete with epi-kal2 for binding to human pKal if binding of the pKal-epi-kal2 complex to anti-pKal antibodies was significantly reduced (>70%) as compared to an injection of hpKal only. The sensor chip surface was regenerated with a pulse of 10 mM glycine pH 1.5 at a flow rate of 100 μl/min Measurements were performed at 25° C. using HBS-P (10 mM HEPES pH 7.4, 150 mM NaCl and 0.005% surfactant P20) as the running buffer. Results from the Biacore competition analysis for epi-Kal2 are shown herein in FIGS. **11**A and **11**B.

Biacore Competition Analysis with the Small Molecule Kallikrein Inhibitor, AEBSF.

AEBSF (i.e., 4-(2-aminoethyl)benzene sulfonyl fluoride hydrochloride) is a small molecule inhibitor of kallikrein. The Biacore competition analysis is used herein to determine whether a test antibody binds to the same site (or an overlapping site) utilized by AEBSF for kallikrein inhibition.

Goat anti-human Fc fragment specific IgG or anti-human Fab IgG was immobilized by amine coupling on a CM5 sensor chip at immobilization densities of approximately 5000 RU. Anti-pKal IgGs or sFabs were captured on their respective surfaces by injecting a 50 nM solution of IgG/sFab for 1-2 minutes at 5 at μl/min Human pKal (100 nM) or human pKal-AEBSF complex (100 nM hpKal that had been pre-treated with 1 mM AEBSF for 1 hour at room temperature) were injected over the captured IgGs or sFabs for 5 minutes at 20-50 μl/min followed by a 5-10 minute dissociation phase. Binding responses were recorded at the end of the association phase. Anti-pKal IgGs or sFabs were considered to compete with AEBSF for binding to human pKal if binding of the pKal-AEBSF complex to anti-pKal antibodies was significantly reduced (>70%) as compared to an injection of hpKal only. The sensor chip surface was regenerated with a pulse of 10 mM glycine pH 1.5 at a flow rate of 100 μl/min. Measurements were performed at 25° C. using HBS-P (10 mM HEPES pH 7.4, 150 mM NaCl and 0.005% surfactant P20) as the running buffer. Results from the Biacore competition analysis for AEBSF are shown herein in FIG. **12**.

The Following are Sequences for the Light Chain Variable Regions (LV), and Heavy Chain Variable Regions (HV) Regions for 8 Exemplary Affinity Matured Anti-pKal Antibodies:

559A-M0029-D09-LV
QSALTQPPTVSVSPGQTARITCSGNKLGDKYVAWYQQKPGQSPMLVIYQDTKRPSRVSERFSGSNSANTAT
LSISGTQALDEADYYCQAWDSSIVIFGGGTRLTVL

559A-M0145-D11-LV
QSVLTQPPSVSVSPGQTASITCSGDKLGDKYTSWYQQRPGQSPVLVIYQDIKRPSGIPERFSGSNSGNTAT
LTISGTQAMDEADYYCQAWDSPNARVFGSGTKVTVL

559A-M0162-A04-LV
DIQMTQSPSTLSASVGDRVTITCRASQSISSWLAWYQQKPGKAPNLLIYKASTLESGVPSRFSGSGSGTEF
TLTISSLQPDDFATYYCQQYNTYWTFGQGTKVEIK

559A-X0101-A01-LV
EIVLTQSPGTLSLSPGERATLSCRTSQFVNSNYLAWYQQKPGQAPRLLIYGASSRATGIPDRFSGSGSGTD
FTLTISRLEPEDFAVYYCQQSSRTPWTFGQGTKVEIK

559A-X0115-A03-LV
DIQMTQSPSTLSASVGDRVTITCRASQSISSWLAWYQQKPGKAPKLLIYKASTLESGVPSRFSGSGSGTEF
TLTISSLQPDDFATYYCQQYNTYWTFGQGTKVEIK

559A-X0115-B07-LV
EIVLTQSPGTLSLSPGERATLSCRTSQFVNSNYLAWYQQKPGQAPRLLIYGASSRATGIPDRFSGSGSGTD
FTLTISRLEPEDFAVYYCQQSSRTPWTFGQGTKVEIK

559A-X0115-D01-LV
DIQMTQSPSTLSASVGDRVTITCRASQSISSWLAWYQQKPGKAPKLLIYKASTLESGVPSRFSGSGSGTEF
TLTISSLQPDDFATYYCQQYNTYWTFGQGTKVEIK

559A-X0115-D05-LV
EIVLTQSPGTLSLSPGERATLSCRTSQFVNSNYLAWYQQKPGQAPRLLIYGASSRATGIPDRFSGSGSGTD
FTLTISRLEPEDFAVYYCQQSSRTPWTFGQGTKVEIK

559A-X0115-E09-LV
EIVLTQSPGTLSLSPGERATLSCRTSQFVNSNYLAWYQQKPGQAPRLLIYGASSRATGIPDRFSGSGSGTD
FTLTISRLEPEDFAVYYCQQSSRTPWTFGQGTKVEIK

559A-X0115-F02-LV
DIQMTQSPSTLSASVGDRVTITCRASQSISSWLAWYQQKPGKAPKLLIYKASTLESGVPSRFSGSGSGTEF
TLTISSLQPDDFATYYCQQYNTYWTFGQGTKVEIK

559A-X0124-G01-LV
DIQMTQSPSTLSASVGDRVTITCRASQSISSWLAWYQQKPGKAPKLLIYKASTLESGVPSRFSGSGSGTEF
TLTISSLQPDDFATYYCQQYNTYWTFGQGTKVEI

559A-X0115-G04-LV
DIQMTQSPSTLSASVGDRVTITCRASQSISSWLAWYQQKPGKAPKLLIYKASTLESGVPSRFSGSGSGTEF
TLTISSLQPDDFATYYCQQYNTYWTFGQGTKVEIK

559A-X0115-H06-LV
EIVLTQSPGTLSLSPGERATLSCRTSQFVNSNYLAWYQQKPGQAPRLLIYGASSRATGIPDRFSGSGSGTD
FTLTISRLEPEDFAVYYCQQSSRTPWTFGQGTKVEIK

559A-M0006-D09-LV
DIQMTQSPSSLSASVGDRVTITCRASQSIRNYLNWYQQKPGKAPNLLIYAASTLQSGVPARFSGSGSGTDF
TLTISSLQPEDFATYYCQQLSGYPHTFGQGTKLEIK

559A-M0035-G04-LV
QDIQMTQSPATLSLSPGERATLSCRASQSVSSYLAWYQQKPGQAPRLLIYDASNRATGIPARFSGSGSGTD
FTLTISSLEPEDFAVYYCQQRSNWPRGFTFGPGTKVDIK

559A-M0029-D09-HV
EVQLLESGGGLVQPGGSLRLSCAASGFTFSWYTMVWVRQAPGKGLEWVSYIYPSGGATFYADSVKGRFTIS
RDNSKNTLYLQMNSLRAEDTAVYYCAMGSYDYIWGFYSDHWGQGTLVTVSS

559A-M0145-D11-HV
EVQLLESGGGLVQPGGSLRLSCAASGFTFSHYRMSWVRQAPGKGLEWVSSIYPSGGRTVYADSVKGRFTIS
RDNSKNTLYLQMNSLRAEDTAVYYCAKDKFEWRLLFRGIGNDAFDIWGQGTMVTVSS

559A-M0162-A04-HV
EVQLLESGGGLVQPGGSLRLSCAASGFTFSHYIMMWVRQAPGKGLEWVSGIYSSGGITVYADSVKGRFTIS
RDNSKNTLYLQMNSLRAEDTAVYYCAYRRTGIPRRDAFDIWGQGTMVTVSS

559A-X0101-A01-HV
EVQLLESGGGLVQPGGSLRLSCAASGFTFSHYLMTWVRQAPGKGLEWVSYISPSGGHTIYADSVKGRFTIS
RDNSKNTLYLQMNSLRAEDTAVYYCARVARGIAARSRTSYFDYWGQGTLVTVSS

-continued

559A-X0115-A03-HV
EVQLLESGGGLVQPGGSLRLSCAASGFTFSHYIMMWVRQAPGKGLEWVSGIYSSGGITVYADSVKGRFTIS
RDNSKNTLYLQMNSLRAEDTAVYYCAWRRIGVPRRDSFDMWGQGTMVTVSS

559A-X0115-B07-HV
EVQLLESGGGLVQPGGSLRLSCAASGFTFSHYLMTWVRQAPGKGLEWVSYISPSGGHTIYADSVKGRFTIS
RDNSKNTLYLQMNSLRAEDTAVYYCAMVGQGIRGRSRTSYFAQWGQGTLVTVSS

559A-X0115-D01-HV
EVQLLESGGGLVQPGGSLRLSCAASGFTFSIYSMHWVRQAPGKGLEWVSSIYPSRGMTWYADSVKGRFTIS
RDNSKNTLYLQMNSLRAEDTAVYYCAYRRTGIPRRDAFDIWGQGTMVTVSS

559A-X0115-D05-HV
EVQLLESGGGLVQPGGSLRLSCAASGFTFSDYMMAWVRQAPGKGLEWVSSIVPSGGHTHYADSVKGRFTIS
RDNSKNTLYLQMNSLRAEDTAVYYCARVARG IAARSRT SYFDYWGQGTLVTVSS

559A-X0115-E09-HV
EVQLLESGGGLVQPGGSLRLSCAASGFTFSHYLMTWVRQAPGKGLEWVSYISPSGGHTIYADSVKGRFTIS
RDNSKNTLYLQMNSLRAEDTAVYYCARVAQGIAARSRTSSVDQWGQGTLVTVSS

559A-X0115-F02-HV
EVQLLESGGGLVQPGGSLRLSCAASGFTFSHYIMMWVRQAPGKGLEWVSGIYSSGGITVYADSVKGRFTIS
RDNSKNTLYLQMNSLRAEDTAVYYCAYRRIGVPRRDEFDIWGQGTMVTVSS

559A-X0124-G01-HV
EVQLLESGGGLVQPGGSLRLSCAASGFTFSHYIMMWVRQAPGKGLEWVSGIYSSGGITVYADSVKGRFTIS
RDNSKNTLYLQMNSLRAEDTAVYYCAYRRIGVPRRDEFDIWGQGTMVTVSS

559A-X0115-G04-HV
EVQLLESGGGLVQPGGSLRLSCAASGFTFSHYIMMWVRQAPGKGLEWVSGIYSSGGITVYADSVKGRFTIS
RDNSKNTLYLQMNSLRAEDTAVYYCAYRRTGVPRRDEFDIWGQGTMVTVSS

559A-X0115-H06-HV
EVQLLESGGGLVQPGGSLRLSCAASGFTFSHYLMTWVRQAPGKGLEWVSYISPSGGHTIYADSVKGRFTIS
RDNSKNTLYLQMNSLRAEDTAVYYCARVAQGISARSRTSYFDYWGQGTLVTVSS

559A-M0006-D09-HV
EVQLLESGGGLVQPGGSLRLSCAASGFTFSFYYMVWVRQAPGKGLEWVSVIYPSGGITVYADSVKGRFTIS
RDNSKNTLYLQMNSLRAEDTAVYYCARDKWAVMPPYYYYAMDVWGQGTTVTVSS

559A-M0035-G04-HV
EVQLLESGGGLVQPGGSLRLSCAASGFTFSYYHMSWVRQAPGKGLEWVSVISPSGGSTKYADSVKGRFTIS
RDNSKNTLYLQMNSLRAEDTAVYYCARGGSSDYAWGSYRRPYYFDYWGQGTLVTVSS

559A-M0029-D09 LV
CAGAGCGCTTTGACTCAGCCACCCACAGTGTCTGTGTCCCCAGGACAGACAGCCAGGATCACCTGCTCTGG
AAATAAATTGGGGGATAAATATGTTGCCTGGTATCAGCAGAAGCCAGGCCAGTCCCCTATGTTGGTCATCT
ATCAAGATACTAAGCGCCCCTCAAGAGTTTCTGAGCGATTCTCTGGCTCCAACTCTGCGAATACAGCCACT
CTGTCCATCAGCGGGACCCAGGCTCTGGATGAGGCTGACTATTACTGTCAGGCGTGGGACAGCAGCATTGT
GATCTTCGGCGGAGGGACCAGGCTGACCGTCCTA

559A-M0145-D11 LV
CAGAGCGTCTTGACTCAGCCACCCTCAGTGTCCGTGTCTCCAGGACAGACAGCCAGCATCACCTGCTCTGG
AGATAAATTGGGGGATAAATATACTTCCTGGTATCAGCAGAGGCCAGGCCAGTCCCCTGTATTGGTCATCT
ATCAAGATATCAAGCGGCCCTCAGGGATCCCTGAGCGATTCTCTGGCTCCAACTCTGGGAACACAGCCACT
CTGACCATCAGCGGGACCCAGGCTATGGATGAGGCTGACTATTACTGTCAGGCGTGGGACAGTCCCAATGC
GAGGGTCTTCGGATCTGGGACCAAGGTCACCGTCCTA

559A-M0162-A04 LV
GACATCCAGATGACCCAGTCTCCTTCCACCCTGTCTGCATCTGTAGGAGACAGAGTCACCATCACTTGCCG
GGCCAGTCAGAGTATCAGTAGTTGGTTGGCCTGGTATCAGCAGAAACCAGGGAAAGCCCCTAACCTCCTGA
TCTATAAGGCGTCTACTTTAGAAAGTGGGGTCCCATCAAGGTTCAGCGGCAGTGGATCTGGGACAGAATTC
ACTCTCACCATCAGCAGCCTGCAGCCTGATGATTTTGCAACTTATTACTGCCAACAGTATAATACTTATTG
GACGTTCGGCCAAGGGACCAAGGTGGAAATCAAA

559A-X0101-A01 LV
GAGATCGTGCTGACCCAGTCCCCTGGCACCCTGTCTCTGTCTCCCGGCGAGAGAGCCACCCTGTCCTGCCG
GACCTCCCAGTTCGTGAACTCCAACTACCTGGCTTGGTATCAGCAGAAGCCAGGCCAGGCCCCTAGACTGC
TGATCTACGGCGCCTCTTCCAGAGCCACCGGCATCCCTGACCGGTTCTCCGGCTCTGGCTCCGGCACCGAC
TTCACCCTGACCATCTCCCGGCTGGAACCTGAGGACTTCGCCGTGTACTACTGCCAGCAGTCCTCCCGGAC
CCCTTGGACCTTTGGCCAGGGCACCAAGGTGGAGATCAAG

559A-X0115-A03 LV
GACATCCAGATGACCCAGTCCCCCTCCACCCTGTCCGCCTCTGTGGGCGACAGAGTGACCATCACCTGTCG
GGCCTCCCAGTCCATCTCCAGCTGGCTGGCCTGGTATCAGCAGAAGCCCGGCAAGGCCCCCAAGCTGCTGA
TCTACAAGGCCAGCACCCTGGAATCCGGCGTGCCCTCCAGATTCTCCGGCTCTGGCTCCGGCACCGAGTTC
ACCCTGACCATCAGCTCCCTGCAGCCCGACGACTTCGCCACCTACTACTGCCAGCAGTACAACACCTACTG
GACCTTCGGCCAGGGCACCAAGGTGGAAATCAAG

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559A-X0115-B07 LV
GAGATCGTGCTGACCCAGTCCCCTGGCACCCTGTCTCTGTCTCCCGGCGAGAGAGCCACCCTGTCCTGCCG
GACCTCCCAGTTCGTGAACTCCAACTACCTGGCTTGGTATCAGCAGAAGCCAGGCCAGGCCCCTAGACTGC
TGATCTACGGCGCCTCTTCCAGAGCCACCGGCATCCCTGACCGGTTCTCCGGCTCTGGCTCCGGCACCGAC
TTCACCCTGACCATCTCCCGGCTGGAACCTGAGGACTTCGCCGTGTACTACTGCCAGCAGTCCTCCCGGAC
CCCTTGGACCTTTGGCCAGGGCACCAAGGTGGAGATCAAG

559A-X0115-D01 LV
GACATCCAGATGACCCAGTCCCCCTCCACCCTGTCCGCCTCTGTGGGCGACAGAGTGACCATCACCTGTCG
GGCCTCCCAGTCCATCTCCAGCTGGCTGGCCTGGTATCAGCAGAAGCCCGGCAAGGCCCCCAAGCTGCTGA
TCTACAAGGCCAGCACCCTGGAATCCGGCGTGCCCTCCAGATTCTCCGGCTCTGGCTCCGGCACCGAGTTC
ACCCTGACCATCAGCTCCCTGCAGCCCGACGACTTCGCCACCTACTACTGCCAGCAGTACAACACCTACTG
GACCTTCGGCCAGGGCACCAAGGTGGAAATCAAG

559A-X0115-D05 LV
GAGATCGTGCTGACCCAGTCCCCTGGCACCCTGTCTCTGTCTCCCGGCGAGAGAGCCACCCTGTCCTGCCG
GACCTCCCAGTTCGTGAACTCCAACTACCTGGCTTGGTATCAGCAGAAGCCAGGCCAGGCCCCTAGACTGC
TGATCTACGGCGCCTCTTCCAGAGCCACCGGCATCCCTGACCGGTTCTCCGGCTCTGGCTCCGGCACCGAC
TTCACCCTGACCATCTCCCGGCTGGAACCTGAGGACTTCGCCGTGTACTACTGCCAGCAGTCCTCCCGGAC
CCCTTGGACCTTTGGCCAGGGCACCAAGGTGGAGATCAAG

559A-X0115-E09 LV
GAGATCGTGCTGACCCAGTCCCCTGGCACCCTGTCTCTGTCTCCCGGCGAGAGAGCCACCCTGTCCTGCCG
GACCTCCCAGTTCGTGAACTCCAACTACCTGGCTTGGTATCAGCAGAAGCCAGGCCAGGCCCCTAGACTGC
TGATCTACGGCGCCTCTTCCAGAGCCACCGGCATCCCTGACCGGTTCTCCGGCTCTGGCTCCGGCACCGAC
TTCACCCTGACCATCTCCCGGCTGGAACCTGAGGACTTCGCCGTGTACTACTGCCAGCAGTCCTCCCGGAC
CCCTTGGACCTTTGGCCAGGGCACCAAGGTGGAGATCAAG

559A-X0115-F02 LV
GACATCCAGATGACCCAGTCCCCCTCCACCCTGTCCGCCTCTGTGGGCGACAGAGTGACCATCACCTGTCG
GGCCTCCCAGTCCATCTCCAGCTGGCTGGCCTGGTATCAGCAGAAGCCCGGCAAGGCCCCCAAGCTGCTGA
TCTACAAGGCCAGCACCCTGGAATCCGGCGTGCCCTCCAGATTCTCCGGCTCTGGCTCCGGCACCGAGTTC
ACCCTGACCATCAGCTCCCTGCAGCCCGACGACTTCGCCACCTACTACTGCCAGCAGTACAACACCTACTG
GACCTTCGGCCAGGGCACCAAGGTGGAAATCAAG

559A-X0115-G04 LV
GACATCCAGATGACCCAGTCCCCCTCCACCCTGTCCGCCTCTGTGGGCGACAGAGTGACCATCACCTGTCG
GGCCTCCCAGTCCATCTCCAGCTGGCTGGCCTGGTATCAGCAGAAGCCCGGCAAGGCCCCCAAGCTGCTGA
TCTACAAGGCCAGCACCCTGGAATCCGGCGTGCCCTCCAGATTCTCCGGCTCTGGCTCCGGCACCGAGTTC
ACCCTGACCATCAGCTCCCTGCAGCCCGACGACTTCGCCACCTACTACTGCCAGCAGTACAACACCTACTG
GACCTTCGGCCAGGGCACCAAGGTGGAAATCAAG

559A-X0115-H06 LV
GAGATCGTGCTGACCCAGTCCCCTGGCACCCTGTCTCTGTCTCCCGGCGAGAGAGCCACCCTGTCCTGCCG
GACCTCCCAGTTCGTGAACTCCAACTACCTGGCTTGGTATCAGCAGAAGCCAGGCCAGGCCCCTAGACTGC
TGATCTACGGCGCCTCTTCCAGAGCCACCGGCATCCCTGACCGGTTCTCCGGCTCTGGCTCCGGCACCGAC
TTCACCCTGACCATCTCCCGGCTGGAACCTGAGGACTTCGCCGTGTACTACTGCCAGCAGTCCTCCCGGAC
CCCTTGGACCTTTGGCCAGGGCACCAAGGTGGAGATCAAG

559A-M0006-D09 LV
GACATCCAGATGACCCAGTCTCCATCCTCCCTGTCTGCATCTGTAGGAGACAGAGTCACCATCACTTGCCG
GGCAAGTCAGAGTATTCGCAACTATTTAAATTGGTATCAGCAGAAACCAGGGAAAGCCCCTAACCTCCTGA
TCTATGCTGCATCCACTTTGCAAAGTGGGGTCCCAGCAAGGTTCAGTGGCAGTGGATCTGGGACAGATTTC
ACTCTCACTATCAGCAGTCTGCAGCCTGAAGATTTTGCAACTTATTACTGTCAACAGCTTAGTGGTTACCC
CCACACTTTTGGCCAGGGGACCAAGCTGGAGATCAAA

559A-M0035-G04 LV
CAAGACATCCAGATGACCCAGTCTCCAGCCACCCTGTCTTTGTCTCCAGGGGAAAGAGCCACCCTCTCCTG
CAGGGCCAGTCAGAGTGTTAGCAGCTACTTAGCCTGGTACCAACAGAAACCTGGCCAGGCTCCCAGGCTCC
TCATCTATGATGCATCCAACAGGGCCACTGGCATCCCAGCCAGGTTCAGTGGCAGTGGGTCTGGGACAGAC
TTCACTCTCACCATCAGCAGCCTAGAGCCTGAAGATTTTGCAGTTTATTACTGTCAGCAGCGTAGCAACTG
GCCTCGCGGATTCACTTTCGGCCCTGGGACCAAAGTGGATATCAAA

559A-M0029-D09 HV
GAAGTTCAATTGTTAGAGTCTGGTGGCGGTCTTGTTCAGCCTGGTGGTTCTTTACGTCTTTCTTGCGCTGC
TTCCGGATTCACTTTCTCTTGGTACACTATGGTTTGGGTTCGCCAAGCTCCTGGTAAAGGTTTGGAGTGGG
TTTCTTATATCTATCCTTCTGGTGGCGCTACTTTTTATGCTGACTCCGTTAAAGGTCGCTTCACTATCTCT
AGAGACAACTCTAAGAATACTCTCTACTTGCAGATGAACAGCTTAAGGGCTGAGGACACGGCCGTGTATTA
CTGTGCGATGGGTTCATATGATTACATTTGGGGATTTTATAGTGACCACTGGGGCCAGGGAACCCTGGTCA
CCGTCTCAAGC

559A-M0145-D11 HV
GAAGTTCAATTGTTAGAGTCTGGTGGCGGTCTTGTTCAGCCTGGTGGTTCTTTACGTCTTTCTTGCGCTGC
TTCCGGATTCACTTTCTCTCATTACCGTATGTCTTGGGTTCGCCAAGCTCCTGGTAAAGGTTTGGAGTGGG
TTTCTTCTATCTATCCTTCTGGTGGCCGTACTGTTTATGCTGACTCCGTTAAAGGTCGCTTCACTATCTCT
AGAGACAACTCTAAGAATACTCTCTACTTGCAGATGAACAGCTTAAGGGCTGAGGACACGGCCGTGTATTA
CTGTGCGAAAGATAAGTTCGAGTGGAGGTTATTATTTCGCGGGATTGGAAATGATGCTTTTGATATCTGGG
GCCAAGGGACAATGGTCACCGTCTCAAGC

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559A-M0162-A04 HV
GAAGTTCAATTGTTAGAGTCTGGTGGCGGTCTTGTTCAGCCTGGTGGTTCTTTACGTCTTTCTTGCGCTGC
TTCCGGATTCACTTTCTCTCATTACATTATGATGTGGGTTCGCCAAGCTCCTGGTAAAGGTTTGGAGTGGG
TTTCTGGTATCTATTCTTCTGGTGGCATTACTGTTTATGCTGACTCCGTTAAAGGTCGCTTCACTATCTCT
AGAGACAACTCTAAGAATACTCTCTACTTGCAGATGAACAGCTTAAGGGCTGAGGACACGGCCGTGTATTA
CTGTGCGTACCGCCGGACTGGGATTCCAAGAAGAGATGCTTTTGATATCTGGGGCCAAGGGACAATGGTCA
CCGTCTCAAGC

559A-X0101-A01 HV
GAGGTGCAATTGCTGGAATCCGGCGGAGGTCTGGTGCAGCCTGGCGGCTCCCTGAGACTGTCTTGCGCCGC
CTCCGGCTTCACCTTCTCCCACTACCTGATGACCTGGGTGCGCCAGGCTCCTGGCAAGGGCCTCGAATGGG
TGTCCTACATCTCCCCCTCTGGCGGCCACACCATCTACGCCGACTCCGTGAAGGGCCGGTTCACCATCTCC
CGGGACAACTCCAAGAACACCCTGTATCTGCAGATGAACTCCCTGAGGGCCGAGGACACCGCCGTGTACTA
CTGCGCCAGGGTGGCCAGAGGAATCGCCGCCAGGTCCCGGACCTCCTACTTCGACTACTGGGGCCAGGGCA
CCCTGGTGACCGTGTCCTCC

559A-X0115-A03 HV
GAGGTGCAATTGCTGGAATCCGGCGGAGGACTGGTGCAGCCTGGCGGCTCCCTGAGACTGTCTTGCGCCGC
CTCCGGCTTTACCTTCTCCCACTACATCATGATGTGGGTGCGACAGGCTCCAGGCAAGGGCCTGGAATGGG
TGTCCGGCATCTACTCCTCCGGCGGCATCACCGTGTACGCCGACTCCGTGAAGGGCCGGTTCACCATCTCC
CGGGACAACTCCAAGAACACCCTGTACCTGCAGATGAACTCCCTGCGGGCCGAGGACACCGCCGTGTACTA
CTGTGCCTGGCGGAGAATCGGCGTGCCCAGACGGGACTCCTTCGACATGTGGGGACAGGGCACCATGGTGA
CAGTGTCCTCC

559A-X0115-B07 HV
GAGGTGCAATTGCTGGAATCCGGCGGAGGACTGGTGCAGCCTGGCGGCTCCCTGAGACTGTCTTGCGCCGC
CTCCGGCTTCACCTTCTCCCACTACCTGATGACCTGGGTGCGACAGGCTCCTGGCAAAGGCCTGGAATGGG
TGTCCTACATCTCCCCCTCTGGCGGCCACACCATCTACGCCGACTCCGTGAAGGGCCGGTTTACCATCTCC
CGGGACAACTCCAAGAACACCCTGTACCTGCAGATGAACTCCCTGCGGGCCGAGGACACCGCCGTGTACTA
CTGTGCCATGGTCGGCCAGGGAATCCGGGGCAGATCCCGGACCTCCTACTTCGCCCAGTGGGGCCAGGGCA
CCCTGGTGACAGTGTCCTCT

559A-X0115-D01 HV
GAGGTGCAATTGCTGGAATCCGGCGGAGGACTGGTGCAGCCTGGCGGCTCCCTGAGACTGTCTTGCGCCGC
CTCCGGCTTCACCTTCTCCATCTACTCCATGCACTGGGTGCGACAGGCTCCAGGCAAGGGCCTGGAATGGG
TGTCCTCCATCTACCCCTCCCGGGGCATGACTTGGTACGCCGACTCCGTGAAGGGCCGGTTCACAATCTCC
CGGGACAACTCCAAGAACACCCTGTACCTGCAGATGAACTCCCTGCGGGCCGAGGACACCGCCGTGTACTA
CTGCGCCTACCGGCGGACCGGCATCCCTAGACGGGACGCCTTCGACATCTGGGGGCAGGGCACCATGGTGA
CAGTGTCCTCC

559A-X0115-D05 HV
GAGGTGCAATTGCTGGAATCCGGCGGTGGACTGGTGCAGCCTGGCGGCTCCCTGAGACTGTCTTGCGCCGC
CTCCGGCTTCACCTTCTCCGACTACATGATGGCCTGGGTGCGACAGGCCCCTGGCAAGGGACTGGAATGGG
TGTCCTCCATCGTGCCCTCTGGCGGCCACACCCACTACGCCGACTCCGTGAAGGGCCGGTTCACCATCTCC
CGGGACAACTCCAAGAACACCCTGTACCTGCAGATGAACTCCCTGCGGGCCGAGGACACCGCCGTGTACTA
CTGCGCCAGAGTGGCCAGAGGAATCGCCGCCAGATCCCGGACCTCCTACTTCGACTACTGGGGCCAGGGCA
CCCTGGTGACAGTGTCCTCC

559A-X0115-E09 HV
GAGGTGCAATTGCTGGAATCCGGCGGAGGACTGGTGCAGCCTGGCGGCTCCCTGAGACTGTCTTGCGCCGC
CTCCGGCTTCACCTTCTCCCACTACCTGATGACCTGGGTGCGACAGGCTCCTGGCAAAGGCCTGGAATGGG
TGTCCTACATCTCCCCCTCTGGCGGCCACACCATCTACGCCGACTCCGTGAAGGGCCGGTTTACCATCTCC
CGGGACAACTCCAAGAACACCCTGTACCTGCAGATGAACTCCCTGCGGGCCGAGGACACCGCCGTGTACTA
CTGTGCCCGGGTGGCCCAGGGAATCGCCGCCAGATCCCGGACCTCCTCTGTGGATCAGTGGGGCCAGGGCA
CCCTGGTGACAGTGTCCTCT

559A-X0115-F02 HV
GAGGTGCAATTGCTGGAATCCGGCGGAGGACTGGTGCAGCCTGGCGGCTCCCTGAGACTGTCTTGCGCCGC
CTCCGGCTTCACCTTCTCCCACTACATCATGATGTGGGTGCGACAGGCTCCTGGCAAGGGGCTGGAATGGG
TGTCCGGCATCTACTCCTCCGGCGGCATCACCGTGTACGCCGACTCCGTGAAGGGCCGGTTCACCATCTCT
CGGGACAACTCCAAGAACACCCTGTACCTGCAGATGAACTCCCTGCGGGCCGAGGACACCGCCGTGTACTA
CTGCGCCTACCGGCGGATCGGCGTGCCCAGACGGGACGAGTTCGACATCTGGGGGCAGGGCACCATGGTGA
CAGTGTCCTCC

559A-X0115-G04 HV
GAGGTGCAATTGCTGGAATCCGGCGGAGGACTGGTGCAGCCTGGCGGCTCCCTGAGACTGTCTTGCGCCGC
CTCCGGCTTCACCTTCTCTCACTACATTATGATGTGGGTGCGACAGGCTCCTGGCAAAGGCCTGGAATGGG
TGTCCGGCATCTACTCCTCCGGCGGCATCACCGTGTACGCCGACTCCGTGAAGGGCCGGTTCACCATCTCC
CGGGACAACTCCAAGAACACCCTGTACCTGCAGATGAACTCCCTGCGGGCCGAGGACACCGCCGTGTACTA
CTGCGCCTACAGACGGACCGGCGTGCCCAGACGGGACGAGTTCGATATCTGGGGGCAGGGCACCATGGTGA
CAGTGTCCTCC

559A-X0115-H06 HV
GAGGTGCAATTGCTGGAATCCGGCGGAGGACTGGTGCAGCCTGGCGGCTCCCTGAGACTGTCTTGCGCCGC
CTCCGGCTTCACCTTCTCCCACTACCTGATGACCTGGGTGCGACAGGCTCCTGGCAAAGGCCTGGAATGGG
TGTCCTACATCTCCCCCTCTGGCGGCCACACCATCTACGCCGACTCCGTGAAGGGCCGGTTTACCATCTCC
CGGGACAACTCCAAGAACACCCTGTACCTGCAGATGAACTCCCTGCGGGCCGAGGACACCGCCGTGTACTA
CTGTGCCCGGGTGGCCCAGGGAATCTCCGCCAGATCCCGGACCTCCTACTTCGATTACTGGGGCCAGGGCA
CCCTGGTGACAGTGTCCTCT

-continued

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559A-M0006-D09 HV
GAAGTTCAATTGTTAGAGTCTGGTGGCGGTCTTGTTCAGCCTGGTGGTTCTTTACGTCTTTCTTGCGCTGC
TTCCGGATTCACTTTCTCTTTTTACTATATGGTTTGGGTTCGCCAAGCTCCTGGTAAAGGTTTGGAGTGGG
TTTCTGTTATCTATCCTTCTGGTGGCATTACTGTTTATGCTGACTCCGTTAAAGGTCGCTTCACTATCTCT
AGAGACAACTCTAAGAATACTCTCTACTTGCAGATGAACAGCTTAAGGGCTGAGGACACGGCCGTGTATTA
CTGTGCGAGAGATAAATGGGCGGTGATGCCCCCCTACTACTACTACGCTATGGACGTCTGGGGCCAAGGGA
CCACGGTCACCGTCTCAAGC

559A-M0035-G04 HV
GAAGTTCAATTGTTAGAGTCTGGTGGCGGTCTTGTTCAGCCTGGTGGTTCTTTACGTCTTTCTTGCGCTGC
TTCCGGATTCACTTTCTCTTATTACCATATGTCTTGGGTTCGCCAAGCTCCTGGTAAAGGTTTGGAGTGGG
TTTCTGTTATCTCTCCTTCTGGTGGCTCTACTAAGTATGCTGACTCCGTTAAAGGTCGCTTCACTATCTCT
AGAGACAACTCTAAGAATACTCTCTACTTGCAGATGAACAGCTTAAGGGCTGAGGACACTGCAGTCTACTA
TTGTGCGAGAGGCGGTTCGAGCGATTACGCTTGGGGGAGTTATCGTCGACCCTACTACTTTGACTACTGGG
GCCAGGGAACCCTGGTCACCGTCTCAAGC
```

Example 13

URP Fusion Proteins of Plasma Kallikrein Binding Proteins

Table 19 shows an annotated sequence of the vector pM160G12URP12 that, in *E. coli*, can cause the secretion of the light chain (LC) of M160-G12 fused to URP1 and the heavy chain (HC) of M160-G12 fused to URP2. In Table 19, the numbered DNA sequence is accompanied with comments, which are denoted on each line following an exclamation point (!). The URPs have no secondary structure in the amino-acid sequence. These sequences are derived from the digits of pi.

URP1 is derived from the first 420 digits of pi. If I is a digit in pi and J is the next digit, then IM=1+integer ((10*I+j)/16). If IM is 1 or 7, the next AA is Gly, IM=2 gives Ala, 3 gives Ser, 4 gives Thr, 5 gives Glu, and 6 gives Pro. URP2 uses digits 421-840. Table 20 contains the unannotated sequence of pM160G12URP12. Table 21 gives the amino-acid sequence of LC(M160-G12)::URP1. Table 22 shows DNA that encodes HC(M160-G12)::URP2. Table 23 shows the amino-acid sequence of HC(M160-G12)::URP2.

Tables 19-23 all show the plasma kallikrein inhibiting Fab of M160-G12, an exemplary plasma kallikrein. It is contemplated herein that any of the antibodies described herein can be put into this or a similar construction. In addition, other sequences could be used for the URPs. In particular, the antibodies M162-A04, M142-H08, X63-G06, X81-B01, X67-D03, and X67-G04 could be substituted for M160-G12. Sequences from U.S. Pat. No. 7,846,445 (herein incorporated by reference in its entirety) can also be used with the plasma kallikrein binding proteins described herein.

TABLE 19

```
pM160G12URP12, annotated
!559A-M160-G12_0III          5932 bp    DNA     circular
!Input = F:\pKal_Ab\559a-m160-g12_LCHC_03_urpv2.ibi
!559A-M160-G12::URP       5932             CIRCULAR
!  Ngene = 5932
!
! Useful REs (cut MAnoLI fewer than 3 times) 2003.02.04
!
! Non-cutters
!AfeI     AGCgct        AvrII      Cctagg              BamHI     Ggatcc
!BclI     Tgatca        BglII      Agatct              BmgBI     CACgtc
!BsaBI    GATNNnnatc    BsiWI      Cgtacg              BsmI      NGcattc
!BspDI    ATcgat        BspMI      Nnnnnnnnngcaggt     BsrGI     Tgtaca
!BstAPI   GCANNNNntgc   BstBI      TTcgaa              BstZ17I   GTAtac
!BtrI     CACgtg        Ec1136I    GAGctc              EcoRV     GATatc
!FseI     GGCCGGcc      HpaI       GTTaac              NdeI      CAtatg
!NsiI     ATGCAt        PacI       TTAATtaa            PmeI      GTTTaaac
!PmlI     CACgtg        PshAI      GACNNnngtc          RsrII     CGgwccg
!SacI     GAGCTc        SacII      CCGCgg              SalI      Gtcgac
!SbfI     CCTGCAgg      SgfI       GCGATcgc            SnaBI     TACgta
!SphI     GCATGc        Sse8387I   CCTGCAgg            StuI      AGGcct
!SwaI     ATTTaaat      TliI       Ctcgag              XcmI      CCANNNNNnnnntgg
!XhoI     Ctcgag
!
! cutters
! Enzymes that cut more than 5 times.
!AgeI Accggt          6
!BsiHKAI GWGCWc       9
!BsrFI Rccggy        15
!EarI CTCTTCNnnn      6
!Eco57I CTGAAG        7
!Eco0109I RGgnccy     7
!FauI nNNNNNNGCGGG   10
!HgiAI GWGCWc         9
!
! Enzymes that cut from 1 to 5 times.
!
! $ = DAM site, * = DCM site, & = both
!
!BssSI      Ctcgtg                1     12
```

TABLE 19-continued

!-"-	Cacgag	1	1703				
!BspHI	Tcatga	4	43	148	1156	3665$	
!AatII	**GACGTc**	**1**	**65**				
!BciVI	GTATCCNNNNNN	2	140	1667			
!AvaI	Cycgrg	3	319	4010	5628		
!BcgI	gcannnnnntcg	2	461	4021$			
!ScaI	AGTact	4	505	3232	3529	4573	
!PvuI	CGATcg	3	616$	4027$	5176$		
!FspI	TGCgca	2	763	5196			
!BglI	GCCNNNNnggc	5	864	3538	3694	4945	5202
!BpmI	CTGGAG	1	898				
!BsaI	GGTCTCNnnnn	1	916				
!-"-	nnnnngagacc	2	3386				
!AhdI	GACNNNnngtc	2	983	5019*			
!Eam1105I	GACNNNnngtc	2	983	5019*			
!AlwNI	CAGNNNctg	2	1462	2923			
!DrdI	GACNNNNnngtc	3	1768	5562	5831		
!PciI	Acatgt	1	1876				
!SapI	gaagagc	1	1998				
!PvuII	CAGctg	2	2054	5146			
!PflMI	CCANNNNntgg	1	2233				
!HindIII	Aagctt	2	2235	3655			
!ApaLI	Gtgcac	1	2321				
!PflFI	GACNnngtc	3	2340	2377	4197		
!Tth111I	GACNnngtc	3	2340	2377	4197		
!BsmFI	Nnnnnnnnnnnnnnngtccc	1	2485				
!-"-	GGGACNNNNNNNNNNnn	2	2530				
!PpuMI	RGgwccy	3	2498	3024	4587		
!SanDI	GGgwccc	1	2498				
!EcoRI	Gaattc	2	2536	5056			
!PstI	CTGCAg	1	2560				
!HincII	GTYrac	1	2591				
!StyI	Ccwwgg	4	2633	3704	4094	4361	
!BsgI	ctgcac	1	2660				
!-"-	GTGCAG	1	5751				
!BbsI	gtcttc	2	2671	4108			
!BlpI	GCtnagc	1	5868				
!EspI	GCtnagc	1	2868				
!AccI	GTmkac	1	2899				
!SgrAI	CRccggyg	2	2936	3585			
!Acc65I	Ggtacc	1	2971				
!KpnI	GGTACc	1	2971				
!BsmBI	CGTCTCNnnnn	3	3104	4077	5877		
!-"-	Nnnnnngagacg	1	5925				
!Bsu36I	CCtnagg	3	3121	3310	4657*		
!NaeI	GCCggc	3	3148	3699	5416		
!NgoMIV	Gccggc	3	3148	3699	5416		
!EagI	Cggccg	4	3284	3983	4397	4805	
!BspEI	Tccgga	4	3453*	3784	4905	4959	
!SexAI	Accwggt	2	3459*	4665*			
!BseRI	NNnnnnnnnnctcctc	4	3466*	4115	4634	4960*	
!EcoNI	CCTnnnnnagg	3	3604	3832*	4167*		
!AscI	GGcgcgcc	1	3613				
!BssHII	Gcgcgc	1	3614				
!SfiI	GGCCNNNNnggcc	1	3693				
!BtgI	Ccrygg	1	3704				
!DsaI	Ccrygg	1	3704				
!NcoI	Ccatgg	1	3704				
!MfeI	Caattg	1	3718				
!BstXI	CCANNNNNntgg	1	3825*				
!MscI	TGGcca	1	3876				
!XbaI	Tctaga	1	3922				
!AflII	Cttaag	1	3966				
!XmaI	Cccggg	1	4010				
!NruI	TCGcga	1	4030$				
!BstEII	Ggtnacc	1	4071*				
!ApaI	GGGCCc	1	4098				
!BanII	GRGCYc	4	4098	4381	4602	5446	
!Bsp120I	Gggccc	1	4098				
!PspOMI	Gggccc	1	4098				
!NheI	Gctagc	1	4116				
!KasI	Ggcgcc	3	4216	4465	5217		
!NotI	GCggccgc	1	4396				
!SpeI	Actagt	1	5020*				
!MluI	Acgcgt	1	5045				
!BsaAI	YACgtr	1	5519				

TABLE 19-continued

```
!DraIII     CACNNNgtg          1   5519
!PsiI       TTAtaa             1   5647
!-----------------------------------------------------------------------
    1 gacgaaaggg cctcgtgata cgcctatttt tataggttaa tgtcatgata ataatggttt
   61 cttaGACGTC aggtggcact tttcggggaa atgtgcgcgg aacccctatt tgtttatttt
!         AatII.
  121 tctaaataca ttcaaatatg tatccgctca tgagacaata accctgataa atgcttcaat
  181 aatattgaaa aaggaagagt atgagtattc aacatttccg tgtcgccctt attccctttt
  241 ttgcggcatt ttgccttcct gtttttgctc acccagaaac gctggtgaaa gtaaaagatg
  301 ctgaagatca gttgggtgcc cgagtgggtt acatcgaact ggatctcaac agcggtaaga
  361 tccttgagag ttttcgcccc gaagaacgtt ttccaatgat gagcactttt aaagttctgc
  421 tatgtggcgc ggtattatcc cgtattgacg ccgggcaaga gcaactcggt cgccgcatac
  481 actattctca gaatgacttg gttgagtact caccagtcac agaaaagcat cttacggatg
  541 gcatgacagt aagagaatta tgcagtgctg ccataaccat gagtgataac actgcggcca
  601 acttacttct gacaacgatc ggaggaccga aggagctaac cgcttttttg cacaacatgg
  661 gggatcatgt aactcgcctt gatcgttggg aaccggagct gaatgaagcc ataccaaacg
  721 acgagcgtga caccacgatg cctgtagcaa tggcaacaac gttgcgcaaa ctattaactg
  781 gcgaactact tactctagct tcccggcaac aattaataga ctggatggag gcggataaag
  841 ttgcaggacc acttctgcgc tcggcccttc cggctggctg gtttattgct gataaatCTG
!                                                                 BpmI.
  901 GAGccggtga gcgtgggtct cgcggtatca ttgcagcact ggggccagat ggtaagccct
!   BpmI.
  961 cccgtatcgt agttatctac acgacgggga gtcaggcaac tatggatgaa cgaaatagac
 1021 agatcgctga gataggtgcc tcactgatta agcattggta actgtcagac caagtttact
 1081 catatatact ttagattgat ttaaaacttc atttttaatt taaaaggatc taggtgaaga
 1141 tcctttttga taatctcatg accaaaatcc cttaacgtga gttttcgttc cactgagcgt
 1201 cagaccccgt agaaaagatc aaaggatctt cttgagatcc tttttttctg cgcgtaatct
 1261 gctgcttgca aacaaaaaaa ccaccgctac cagcggtggt ttgtttgccg gatcaagagc
 1321 taccaactct ttttccgaag gtaactggct tcagcagagc gcagatacca aatactgttc
 1381 ttctagtgta gccgtagtta ggccaccact tcaagaactc tgtagcaccg cctacatacc
 1441 tcgctctgct aatcctgtta ccagtggctg ctgccagtgg cgataagtcg tgtcttaccg
 1501 ggttggactc aagacgatag ttaccggata aggcgcagcg gtcgggctga acggggggtt
 1561 cgtgcataca gcccagcttg gagcgaacga cctacaccga actgagatac ctacagcgtg
 1621 agctatgaga aagcgccacg cttcccgaag ggagaaaggc ggacaggtat ccggtaagcg
 1681 gcagggtcgg aacaggagag cgcacgaggg agcttccagg gggaaacgcc tggtatcttt
 1741 atagtcctgt cgggtttcgc cacctctgac ttgagcgtcg atttttgtga tgctcgtcag
 1801 gggggcggag cctatggaaa aacgccagca acgcggcctt tttacggttc ctggcctttt
 1861 gctggccttt tgctcACATG Ttctttcctg cgttatcccc tgattctgtg gataaccgta
!                    PciI...
 1921 ttaccgcctt tgagtgagct gataccgctc gccgcagccg aacgaccgag cgcagcgagt
 1981 cagtgagcga ggaagcgGAA GAGCgcccaa tacgcaaacc gcctctcccc gcgcgttggc
!                      SapI....
 2041 cgattcatta atgcagctgg cacgacaggt ttcccgactg gaaagcgggc agtgagcgca
 2101 acgcaattaa tgtgagttag ctcactcatt aggcacccca ggctttacac tttatgcttc
 2161 cggctcgtat gttgtgtgga attgtgagcg gataacaatt tcacacagga aacagctatg
 2221 accatgatta cgccaagctt tggagccttt tttttggaga ttttcaac
!
! M160-G12                LC
!QDIQMTQSPS FLSASVGDRV TITCRASQGI SSYLSWYQQK PGKAPKLLIY AASTLQSGVP  60
!SRFSGSGSGT EFTLTISSLQ PEDFATYYCQ QLNSYPLTFG GGTKVEIK             108
!
!        LC signal sequence----------------------------------------
!         1   2   3   4   5   6   7   8   9  10  11  12  13  14  15
!         M   K   K   L   L   F   A   I   P   L   V   V   P   F   Y
  2269   atg aaa aaa tta tta ttc gca att cct tta gtt gtt cct ttc tat
!
!        Signal------    | LC Vkappa-------------------------------
!        16  17  18  19  20  21  22  23  24  25  26  27  28  29  30
!         S   H   S   A   Q   D   I   Q   M   T   Q   S   P   S   F
  2314   tct cac aGT GCA Caa gac atc cag atg acc cag tct cca tcc ttc
!                 ApaLI...
!
!        LC Vkappa-------------------------------------------------
!        31  32  33  34  35  36  37  38  39  40  41  42  43  44  45
!         L   S   A   S   V   G   D   R   V   T   I   T   C   R   A
  2359   ctg tct gca tct gta gga gac aga gtc acc atc act tgc cgg gcc
!
!        LC Vkappa-------------------------------------------------
!        46  47  48  49  50  51  52  53  54  55  56  57  58  59  60
!         S   Q   G   I   S   S   Y   L   A   W   Y   Q   Q   K   P
  2404   agt cag ggc att agc agt tat tta gcc tgg tat cag caa aaa cca

!        LC Vkappa-------------------------------------------------
!        61  62  63  64  65  66  67  68  69  70  71  72  73  74  75
!         G   K   A   P   K   L   L   I   Y   A   A   S   T   L   Q
  2449   ggg aaa gcc cct aag ctc ctg atc tat gct gca tcc act ttg caa
!
!        LC Vkappa-------------------------------------------------
!        76  77  78  79  80  81  82  83  84  85  86  87  88  89  90
!         S   G   V   P   S   R   F   S   G   S   G   S   G   T   E
```

TABLE 19-continued

```
 2494     agt gGG GTC CCa tca agg ttc agc ggc agt gga tct ggg aca gaa
!              SanDI....
!
!         LC Vkappa-----------------------------------------------------
!          91  92  93  94  95  96  97  98  99 100 101 102 103 104 105
!          F   T   L   T   I   S   S   L   Q   P   E   D   F   A   T
 2539     ttc act ctc aca atc agc agc CTG CAG cct gaa gat ttt gca act
!                                     PstI...
!
!         LC Vkappa-----------------------------------------------------
!         106 107 108 109 110 111 112 113 114 115 116 117 118 119 120
!          Y   Y   C   Q   Q   L   N   S   Y   P   L   T   F   G   G
 2584     tat tac tGT CAA Cag ctt aat agt tac cct ctc act ttc ggc gga
!                 HincII..
!
!         LC Vkappa------------------ | Ckappa-----------------------
!         121 122 123 124 125 126 127 128 129 130 131 132 133 134 135
!          G   T   K   V   E   I   K   R   T   V   A   A   P   S   V
 2629     ggg acc aag gtg gag atc aaa cga act gtg gct gca cca tct gtc
!
!         Ckappa--------------------------------------------------------
!         136 137 138 139 140 141 142 143 144 145 146 147 148 149 150
!          F   I   F   P   P   S   D   E   Q   L   K   S   G   T   A
 2674     ttc atc ttc ccg cca tct gat gag cag ttg aaa tct gga act gcc
!
!         Ckappa--------------------------------------------------------
!         151 152 153 154 155 156 157 158 159 160 161 162 163 164 165
!          S   V   V   C   L   L   N   N   F   Y   P   R   E   A   K
 2719     tct gtt gtg tgc ctg ctg aat aac ttc tat ccc aga gag gcc aaa
!
!         Ckappa--------------------------------------------------------
!         166 167 168 169 170 171 172 173 174 175 176 177 178 179 180
!          V   Q   W   K   V   D   N   A   L   Q   S   G   N   S   Q
 2764     gta cag tgg aag gtg gat aac gcc ctc caa tcg ggt aac tcc cag
!
!         Ckappa--------------------------------------------------------
!         181 182 183 184 185 186 187 188 189 190 191 192 193 194 195
!          E   S   V   T   E   Q   D   S   K   D   S   T   Y   S   L
 2809     gag agt gtc aca gag cag gac agc aag gac agc acc tac agc ctc
!
!         Ckappa--------------------------------------------------------
!         196 197 198 199 200 201 202 203 204 205 206 207 208 209 210
!          S   S   T   L   T   L   S   K   A   D   Y   E   K   H   K
 2854     agc agc acc ctg acG CTG AGC aaa gca gac tac gag aaa cac aaa
!                             BlpI.....
!
!         Ckappa--------------------------------------------------------
!         211 212 213 214 215 216 217 218 219 220 221 222 223 224 225
!          V   Y   A   C   E   V   T   H   Q   G   L   S   S   P   V
 2899     GTC TAC gcc tgc gaa gtc acc cat cag ggc ctg agt tca ccg gtg
!         AccI...
!
!         Ckappa----------------------------- ! URP------------------
!         226 227 228 229 230 231 232 233 234 235 236 237 238 239 240
!          T   K   S   F   N   R   G   E   C   G   T   A   S   T   A
 2944     aca aag agc ttc aac agg gga gag tgt GGT ACC gct tct act gcc
!                                             KpnI...
!
!         URP-----------------------------------------------------------
!         241 242 243 244 245 246 247 248 249 250 251 252 253 254 255
!          T   T   G   P   A   P   T   E   S   P   A   P   G   P   S
 2989     acc act ggt cct gct cct act gaa tcc cct gct ccg ggt cct tct
!
!         URP-----------------------------------------------------------
!         256 257 258 259 260 261 262 263 264 265 266 267 268 269 270
!          G   A   P   G   S   T   G   P   G   E   P   S   P   S   E
 3034     ggt gct cct ggc tct act ggt cct ggt gag ccg agt cct agt gaa
!
!         URP-----------------------------------------------------------
!         271 272 273 274 275 276 277 278 279 280 281 282 283 284 285
!          A   T   T   P   A   P   G   T   P   S   P   T   S   G   P
 3079     gcc acc act cct gct cct ggt act ccg tct cct act tcc ggc cct
!
!         URP-----------------------------------------------------------
!         286 287 288 289 290 291 292 293 294 295 296 297 298 299 300
!          E   G   A   T   G   E   G   A   A   G   E   P   P   P   S
 3124     gag ggt gct acc ggt gaa ggt gct gcc ggc gag cct ccg cct tct
!
!         URP-----------------------------------------------------------
!         301 302 303 304 305 306 307 308 309 310 311 312 313 314 315
```

TABLE 19-continued

```
!         G   T   G   P   A   A   A   S   P   G   G   P   P   G   E
 3169    ggt act ggt cct gct gct gct tct cct ggt ggc ccg cct ggt gaa
!
!        URP---------------------------------------------------------
!        316 317 318 319 320 321 322 323 324 325 326 327 328 329 330
!         T   A   S   G   P   A   S   T   G   G   T   G   S   T   A
 3214    act gcc agt ggt cct gct agt act ggt ggc acc ggt tct act gct
!
!        URP---------------------------------------------------------
!        331 332 333 334 335 336 337 338 339 340 341 342 343 344 345
!         T   P   T   S   S   A   E   S   P   A   G   T   E   P   S
 3259    act cct act tcc tct gct gag tct ccg gcc ggt act gaa cct agt
!
!        URP---------------------------------------------------------
!        346 347 348 349 350 351 352 353 354 355 356 357 358 359 360
!         S   G   P   E   E   P   S   E   E   P   A   T   E   A   A
 3304    agt ggt cct gag gaa cct tct gag gaa ccg gct act gag gct gct
!
!        URP---------------------------------------------------------
!        361 362 363 364 365 366 367 368 369 370 371 372 373 374 375
!         G   G   T   T   T   E   A   S   G   T   T   G   T   S   E
 3349    ggc ggt act act acc gaa gcc tcc ggt act act ggt act tct gag
!
!        URP---------------------------------------------------------
!        376 377 378 379 380 381 382 383 384 385 386 387 388 389 390
!         T   A   S   P   E   E   E   A   P   S   A   S   A   T   P
 3394    acc gct tct cct gaa gag gaa gct cct agt gct agt gcc act cct
!
!        URP---------------------------------------------------------
!        391 392 393 394 395 396 397 398 399 400 401 402 403 404 405
!         G   E   T   G   T   P   E   P   G   A   P   G   T   P   P
 3439    ggc gag act ggt act ccg gaa cct ggt gct cct ggt act cct ccg
!
!        URP---------------------------------------------------------
!        406 407 408 409 410 411 412 413 414 415 416 417 418 419 420
!         T   G   A   G   S   S   E   P   A   G   S   G   G   S   G
 3484    act ggc gct ggt tct tcc gag cct gct ggt tct ggt ggc tct ggt
!
!        URP---------------------------------------------------------
!        421 422 423 424 425 426 427 428 429 430 431 432 433 434 435
!         S   T   P   A   S   E   A   S   S   S   P   A   S   T   A
 3529    agt act cct gcc agt gag gct tct tcc tct cct gct tct act gct
!
!        URP---------------------------------------
!        436 437 438 439 440 441 442 443 444 445 446 447 448
!         G   S   S   T   A   G   E   E   P   P   P   .   .
 3574    ggt agt agt acc gcc ggt gag gaa ccg cct cct taa taa
!
 3613                                GGCG CGCCtaacca tctatttcaa
!                                    AscI.....
!                                     BssHII.
 3637    ggaacagtct ta
!
!M160-G12                  HC
!EVQLLESGGG LVQPGGSLRL SCAASGFTFS HYLMTWVRQA PGKGLEWVSY ISPSGGHTIY  60
!ADSVKGRFTI SRDNSKNTLY LQMNSLRAED TAVYYCARVA RGIAARSRTS YFDYWGQGTL 120
!VTVSSASTKG PSVFPLAPSS KS                                         142

!
!       HC signal sequence  -------------------------------------
!          1   2   3   4   5   6   7   8   9  10  11  12  13  14  15
!         M   K   K   L   L   F   M   I   P   L   V   V   P   F   V
 3649    atg aaa aag ctt tta ttc atg atc ccg tta gtt gta ccg ttc gtG
!                                                    SfiI         .
!
!   signal sequence ------------- |--- VH---------------------------
!         16  17  18  19  20  21  22  23  24  25  26  27  28  29  30
!         A   Q   P   A   M   A   E   V   Q   L   L   E   S   G   G
 3694    GCC CAG CCG GCC atg gcc gaa gtt CAA TTG tta gag tct ggt ggc
!     SfiI..............                MfeI...
!                   NcoI....

!         VH-------------------------------------------------------
!         31  32  33  34  35  36  37  38  39  40  41  42  43  44  45
!         G   L   V   Q   P   G   G   S   L   R   L   S   C   A   A
 3739    ggt ctt gtt cag cct ggt ggt tct tta cgt ctt tct tgc gct gct
!
!         VH-------------------------------------------------------
!         46  47  48  49  50  51  52  53  54  55  56  57  58  59  60
!         S   G   F   T   F   S   H   Y   L   M   T   W   V   R   Q
```

TABLE 19-continued

```
 3784    tcc gga ttc act ttc tct cat tac ctt atg act tgg gtt cgc caa
!
!         61  62  63  64  65  66  67  68  69  70  71  72  73  74  75
!         A   P   G   K   G   L   E   W   V   S   Y   I   S   P   S
 3829    gct cct ggt aaa ggt ttg gag tgg gtt tct tat atc tct cct tct
!
!         VH-----------------------------------------------------
!         76  77  78  79  80  81  82  83  84  85  86  87  88  89  90
!         G   G   H   T   I   Y   A   D   S   V   K   G   R   F   T
 3874    ggt ggc cat act att tat gct gac tcc gtt aaa ggt cgc ttc act
!
!         VH-----------------------------------------------------
!         91  92  93  94  95  96  97  98  99 100 101 102 103 104 105
!         I   S   R   D   N   S   K   N   T   L   Y   L   Q   M   N
 3919    atc TCT AGA gac aac tct aag aat act ctc tac ttg cag atg aac
!            XbaI...
!
!         VH-----------------------------------------------------
!        106 107 108 109 110 111 112 113 114 115 116 117 118 119 120
!         S   L   R   A   E   D   T   A   V   Y   Y   C   A   R   V
 3964    agc tta agg gct gag gac acg gcc gtg tat tac tgt gcg aga gtg
!
!         VH-----------------------------------------------------
!        121 122 123 124 125 126 127 128 129 130 131 132 133 134 135
!         A   R   G   I   A   A   R   S   R   T   S   Y   F   D   Y
 4009    gCC CGG Ggg ata gca gtc cga TCG CGA acc agc tac ttt gac tac
!         XmaI....                   NruI...
!
!         VH-------------------------------------
!        136 137 138 139 140 141 142 143 144 145 146 147 148 149 150
!         W   G   Q   G   T   L   V   T   V   S   S   A   S   T   K
 4054    tgg ggc cag gga acc ctG GTC ACC gtc tca agc gcc tcc acc aaG
!                               BstEII...                  Bsp120I .
!
!        151 152 153 154 155 156 157 158 159 160 161 162 163 164 165
!         G   P   S   V   F   P   L   A   P   S   S   K   S   T   S
 4099    GGC CCa tcg gtc ttc ccG CTA GCa ccc tcc tcc aag agc acc tct
!      Bsp120I.                 NheI....
!
!        166 167 168 169 170 171 172 173 174 175 176 177 178 179 180
!         G   G   T   A   A   L   G   C   L   V   K   D   Y   F   P
 4144    ggg ggc aca gcg gcc ctg ggc tgc ctg gtc aag gac tac ttc ccc
!
!        181 182 183 184 185 186 187 188 189 190 191 192 193 194 195
!         E   P   V   T   V   S   W   N   S   G   A   L   T   S   G
 4189    gaa ccg gtg acg gtg tcg tgg aac tca ggc gcc ctg acc agc ggc
!
!        196 197 198 199 200 201 202 203 204 205 206 207 208 209 210
!         V   H   T   F   P   A   V   L   Q   S   S   G   L   Y   S
 4234    gtc cac acc ttc ccg gct gtc cta cag tct agc gga ctc tac tcc
!
!        211 212 213 214 215 216 217 218 219 220 221 222 223 224 225
!         L   S   S   V   V   T   V   P   S   S   S   L   G   T   Q
 4279    ctc agc agc gta gtg acc gtg ccc tct tct agc ttg ggc acc cag
!
!        226 227 228 229 230 231 232 233 234 235 236 237 238 239 240
!         T   Y   I   C   N   V   N   H   K   P   S   N   T   K   V
 4324    acc tac atc tgc aac gtg aat cac aag ccc agc aac acc aag gtg
!
!                                             URP----------------->
!        241 242 243 244 245 246 247 248 249 250 251 252 253 254 255
!         D   K   K   V   E   P   K   S   C   A   A   A   S   P   A
 4369    gac aag aaa gtt gag ccc aaa tct tgt GCG GCC GCt tct cct gct
!                                             NotI......
!
!        256 257 258 259 260 261 262 263 264 265 266 267 268 269 270
!         T   A   S   A   S   T   A   P   A   T   A   T   P   E   S
 4414    act gct tcc gct tct act gcc ccg gct act gct acc cct gag tct
!
!        271 272 273 274 275 276 277 278 279 280 281 282 283 284 285
!         A   E   G   A   T   T   E   T   P   T   T   E   T   P   A
 4459    gct gaa ggc gcc act act gag act cct acc act gaa act cct gct
!
!        286 287 288 289 290 291 292 293 294 295 296 297 298 299 300
!         E   S   A   S   G   P   P   A   P   S   E   S   A   T   E
 4504    gag agt gct agt ggt ccg cct gct cct tct gaa tcc gcc act gag
!
!        301 302 303 304 305 306 307 308 309 310 311 312 313 314 315
!         E   S   G   E   A   S   T   S   S   T   A   E   E   G   P
 4549    gaa tct ggt gag gct tct acc agt agt act gct gaa gag ggt cct
```

TABLE 19-continued

```
!
!        316 317 318 319 320 321 322 323 324 325 326 327 328 329 330
!         A   E   P   G   S   P   A   P   T   P   A   A   T   P   A
  4594   gct gaa ccg ggc tct cct gcc cct act cct gct gct act ccg gct
!
!        331 332 333 334 335 336 337 338 339 340 341 342 343 344 345
!         E   T   S   S   E   P   P   E   E   P   G   G   A   G   T
  4639   gag acc tcc tct gaa cct cct gag gaa cct ggt ggt gcc ggt act
!
!        346 347 348 349 350 351 352 353 354 355 356 357 358 359 360
!         P   A   G   T   T   T   G   A   E   T   E   S   A   T   E
  4684   ccg gct ggc act act acc ggt gct gag act gaa tct gct act gag
!
!        361 362 363 364 365 366 367 368 369 370 371 372 373 374 375
!         G   G   A   S   S   A   P   A   S   P   T   G   G   A   P
  4729   ggt ggt gcc agt agt gct cct gct tct cct act ggc ggt gct cct
!
!        376 377 378 379 380 381 382 383 384 385 386 387 388 389 390
!         S   S   G   E   T   T   T   E   G   G   P   A   G   P   A
  4774   tcc tct ggt gaa acc act act gag ggt ggc ccg gcc ggt cct gct
!
!        391 392 393 394 395 396 397 398 399 400 401 402 403 404 405
!         P   A   T   A   A   P   T   G   G   G   A   G   G   E   G
  4819   cct gct act gct gcc cct acc ggt ggt ggc gct ggt ggt gaa ggt
!
!        406 407 408 409 410 411 412 413 414 415 416 417 418 419 420
!         S   A   G   G   G   T   G   E   E   G   G   G   G   A   P
  4864   tct gct ggc ggt ggt act ggt gag gaa ggc ggt ggt ggt gct ccg
!
!        421 422 423 424 425 426 427 428 429 430 431 432 433 434 435
!         E   G   S   G   G   G   P   E   G   P   T   P   A   T   E
  4909   gag ggc agt ggt ggt ggt cct gaa ggc cct act cct gcc act gag
!
         436 437 438 439 440 441 442 443 444 445 446 447 448 449 450
!         A   S   P   E   G   A   P   P   G   S   T   S   T   S   G
  4954   gct agt ccg gaa ggt gct cct cct ggt tct acc tcc act tct ggt
!
!        451 452 453 454 455 456 457 458 459 460 461 462 463
!         P   G   E   A   A   S   P   T   S   S   P   G   .
  4999   cct ggc gag gct ggc tct ccg ACT AGT agt cct ggt taa
!                                   SpeI...
!
  5038   tga taa
!
  5044   aACGC GTgatgaga attcactggc cgtcgtttta caacgtcgtg actgggaaaa
!         MluI...
  5098   ccctggcgtt acccaactta atcgccttgc agcacatccc cctttcgcca gctggcgtaa
  5158   tagcgaagag gcccgcaccg atcgcccttc ccaacagttg cgcagcctga atggcgaatg
  5218   gcgcctgatg cggtattttc tccttacgca tctgtgcggt atttcacacc gcatacgtca
  5278   aagcaaccat agtacgcgcc ctgtagcggc gcattaagcg cggcgggtgt ggtggttacg
  5338   cgcagcgtga ccgctacact tgccagcgcc ttagcgcccg ctcctttcgc tttcttccct
  5398   tcctttctcg ccacgttcgc cggctttccc cgtcaagctc taaatcgggg gctcccttta
  5458   gggttccgat ttagtgcttt acggcacctc gaccccaaaa aacttgattt gggtgatggt
  5518   tCACGTAgtg ggccatcgcc ctgatagacg gtttttcgcc ctttgacgtt ggagtccacg
!         BsaAI.
!         DraIII...
  5578   ttctttaata gtggactctt gttccaaact ggaacaacac tcaactctat ctcgggctat
  5638   tcttttgatT TATAAgggat tttgccgatt tcggtctatt ggttaaaaaa tgagctgatt
!                 PsiI...
  5698   taacaaaaat ttaacgcgaa ttttaacaaa atattaacgt ttacaatttt atggtgcagt
  5758   ctcagtacaa tctgctctga tgccgcatag ttaagccagc cccgacaccc gccaacaccc
  5818   gctgacgcgc cctgacgggc ttgtctgctc ccggcatccg cttacagaca agctgtgacc
  5878   gtctccggga gctgcatgtg tcagaggttt tcaccgtcat caccgaaacg cgcga
```

TABLE 20

unannotated DNA sequence of pM160G12:URP12

```
LOCUS       pM160G12  5932               CIRCULAR
!M160-G12_URP1-2     5926 bp    DNA       circular
ORIGIN
    1 GACGAAAGGG CCTCGTGATA CGCCTATTTT TATAGGTTAA TGTCATGATA ATAATGGTTT

   61 CTTAGACGTC AGGTGGCACT TTTCGGGGAA ATGTGCGCGG AACCCCTATT TGTTTATTTT

  121 TCTAAATACA TTCAAATATG TATCCGCTCA TGAGACAATA ACCCTGATAA ATGCTTCAAT
```

TABLE 20-continued

unannotated DNA sequence of pM160G12:URP12

```
 181 AATATTGAAA AAGGAAGAGT ATGAGTATTC AACATTTCCG TGTCGCCCTT ATTCCCTTTT
 241 TTGCGGCATT TTGCCTTCCT GTTTTTGCTC ACCCAGAAAC GCTGGTGAAA GTAAAAGATG
 301 CTGAAGATCA GTTGGGTGCC CGAGTGGGTT ACATCGAACT GGATCTCAAC AGCGGTAAGA
 361 TCCTTGAGAG TTTTCGCCCC GAAGAACGTT TTCCAATGAT GAGCACTTTT AAAGTTCTGC
 421 TATGTGGCGC GGTATTATCC CGTATTGACG CCGGGCAAGA GCAACTCGGT CGCCGCATAC
 481 ACTATTCTCA GAATGACTTG GTTGAGTACT CACCAGTCAC AGAAAAGCAT CTTACGGATG
 541 GCATGACAGT AAGAGAATTA TGCAGTGCTG CCATAACCAT GAGTGATAAC ACTGCGGCCA
 601 ACTTACTTCT GACAACGATC GGAGGACCGA AGGAGCTAAC CGCTTTTTTG CACAACATGG
 661 GGGATCATGT AACTCGCCTT GATCGTTGGG AACCGGAGCT GAATGAAGCC ATACCAAACG
 721 ACGAGCGTGA CACCACGATG CCTGTAGCAA TGGCAACAAC GTTGCGCAAA CTATTAACTG
 781 GCGAACTACT TACTCTAGCT TCCCGGCAAC AATTAATAGA CTGGATGGAG GCGGATAAAG
 841 TTGCAGGACC ACTTCTGCGC TCGGCCCTTC CGGCTGGCTG GTTTATTGCT GATAAATCTG
 901 GAGCCGGTGA GCGTGGGTCT CGCGGTATCA TTGCAGCACT GGGGCCAGAT GGTAAGCCCT
 961 CCCGTATCGT AGTTATCTAC ACGACGGGGA GTCAGGCAAC TATGGATGAA CGAAATAGAC
1021 AGATCGCTGA GATAGGTGCC TCACTGATTA AGCATTGGTA ACTGTCAGAC CAAGTTTACT
1081 CATATATACT TTAGATTGAT TTAAAACTTC ATTTTTAATT TAAAAGGATC TAGGTGAAGA
1141 TCCTTTTTGA TAATCTCATG ACCAAAATCC CTTAACGTGA GTTTTCGTTC CACTGAGCGT
1201 CAGACCCCGT AGAAAAGATC AAAGGATCTT CTTGAGATCC TTTTTTTCTG CGCGTAATCT
1261 GCTGCTTGCA AACAAAAAAA CCACCGCTAC CAGCGGTGGT TTGTTTGCCG GATCAAGAGC
1321 TACCAACTCT TTTTCCGAAG GTAACTGGCT TCAGCAGAGC GCAGATACCA AATACTGTTC
1381 TTCTAGTGTA GCCGTAGTTA GGCCACCACT TCAAGAACTC TGTAGCACCG CCTACATACC
1441 TCGCTCTGCT AATCCTGTTA CCAGTGGCTG CTGCCAGTGG CGATAAGTCG TGTCTTACCG
1501 GGTTGGACTC AAGACGATAG TTACCGGATA AGGCGCAGCG GTCGGGCTGA ACGGGGGGTT
1561 CGTGCATACA GCCCAGCTTG GAGCGAACGA CCTACACCGA ACTGAGATAC CTACAGCGTG
1621 AGCTATGAGA AAGCGCCACG CTTCCCGAAG GGAGAAAGGC GGACAGGTAT CCGGTAAGCG
1681 GCAGGGTCGG AACAGGAGAG CGCACGAGGG AGCTTCCAGG GGGAAACGCC TGGTATCTTT
1741 ATAGTCCTGT CGGGTTTCGC CACCTCTGAC TTGAGCGTCG ATTTTTGTGA TGCTCGTCAG
1801 GGGGGCGGAG CCTATGGAAA AACGCCAGCA ACGCGGCCTT TTTACGGTTC CTGGCCTTTT
1861 GCTGGCCTTT TGCTCACATG TTCTTTCCTG CGTTATCCCC TGATTCTGTG GATAACCGTA
1921 TTACCGCCTT TGAGTGAGCT GATACCGCTC GCCGCAGCCG AACGACCGAG CGCAGCGAGT
1981 CAGTGAGCGA GGAAGCGGAA GAGCGCCCAA TACGCAAACC GCCTCTCCCC GCGCGTTGGC
2041 CGATTCATTA ATGCAGCTGG CACGACAGGT TTCCCGACTG GAAAGCGGGC AGTGAGCGCA
2101 ACGCAATTAA TGTGAGTTAG CTCACTCATT AGGCACCCCA GGCTTTACAC TTTATGCTTC
2161 CGGCTCGTAT GTTGTGTGGA ATTGTGAGCG GATAACAATT TCACACAGGA AACAGCTATG
2221 ACCATGATTA CGCCAAGCTT TGGAGCCTTT TTTTTGGAGA TTTTCAACAT GAAAAAATTA
2281 TTATTCGCAA TTCCTTTAGT TGTTCCTTTC TATTCTCACA GTGCACAAGA CATCCAGATG
2341 ACCCAGTCTC CATCCTTCCT GTCTGCATCT GTAGGAGACA GAGTCACCAT CACTTGCCGG
2401 GCCAGTCAGG GCATTAGCAG TTATTTAGCC TGGTATCAGC AAAAACCAGG GAAAGCCCCT
2461 AAGCTCCTGA TCTATGCTGC ATCCACTTTG CAAAGTGGGG TCCCATCAAG GTTCAGCGGC
```

TABLE 20-continued

unannotated DNA sequence of pM160G12:URP12
2521 AGTGGATCTG GGACAGAATT CACTCTCACA ATCAGCAGCC TGCAGCCTGA AGATTTTGCA
2581 ACTTATTACT GTCAACAGCT TAATAGTTAC CCTCTCACTT TCGGCGGAGG GACCAAGGTG
2641 GAGATCAAAC GAACTGTGGC TGCACCATCT GTCTTCATCT TCCCGCCATC TGATGAGCAG
2701 TTGAAATCTG GAACTGCCTC TGTTGTGTGC CTGCTGAATA ACTTCTATCC CAGAGAGGCC
2761 AAAGTACAGT GGAAGGTGGA TAACGCCCTC CAATCGGGTA ACTCCCAGGA GAGTGTCACA
2821 GAGCAGGACA GCAAGGACAG CACCTACAGC CTCAGCAGCA CCCTGACGCT GAGCAAAGCA
2881 GACTACGAGA AACACAAAGT CTACGCCTGC GAAGTCACCC ATCAGGGCCT GAGTTCACCG
2941 GTGACAAAGA GCTTCAACAG GGGAGAGTGT GGTACCGCTT CTACTGCCAC CACTGGTCCT
3001 GCTCCTACTG AATCCCCTGC TCCGGGTCCT TCTGGTGCTC CTGGCTCTAC TGGTCCTGGT
3061 GAGCCGAGTC CTAGTGAAGC CACCACTCCT GCTCCTGGTA CTCCGTCTCC TACTTCCGGC
3121 CCTGAGGGTG CTACCGGTGA AGGTGCTGCC GGCGAGCCTC CGCCTTCTGG TACTGGTCCT
3181 GCTGCTGCTT CTCCTGGTGG CCCGCCTGGT GAAACTGCCA GTGGTCCTGC TAGTACTGGT
3241 GGCACCGGTT CTACTGCTAC TCCTACTTCC TCTGCTGAGT CTCCGGCCGG TACTGAACCT
3301 AGTAGTGGTC CTGAGGAACC TTCTGAGGAA CCGGCTACTG AGGCTGCTGG CGGTACTACT
3361 ACCGAAGCCT CCGGTACTAC TGGTACTTCT GAGACCGCTT CTCCTGAAGA GGAAGCTCCT
3421 AGTGCTAGTG CCACTCCTGG CGAGACTGGT ACTCCGGAAC CTGGTGCTCC TGGTACTCCT
3481 CCGACTGGCG CTGGTTCTTC CGAGCCTGCT GGTTCTGGTG GCTCTGGTAG TACTCCTGCC
3541 AGTGAGGCTT CTTCCTCTCC TGCTTCTACT GCTGGTAGTA GTACCGCCGG TGAGGAACCG
3601 CCTCCTTAAT AAGGCGCGCC TAACCATCTA TTTCAAGGAA CAGTCTTAAT GAAAAAGCTT
3661 TTATTCATGA TCCCGTTAGT TGTACCGTTC GTGGCCCAGC CGGCCATGGC CGAAGTTCAA
3721 TTGTTAGAGT CTGGTGGCGG TCTTGTTCAG CCTGGTGGTT CTTTACGTCT TTCTTGCGCT
3781 GCTTCCGGAT TCACTTTCTC TCATTACCTT ATGACTTGGG TTCGCCAAGC TCCTGGTAAA
3841 GGTTTGGAGT GGGTTTCTTA TATCTCTCCT TCTGGTGGCC ATACTATTTA TGCTGACTCC
3901 GTTAAAGGTC GCTTCACTAT CTCTAGAGAC AACTCTAAGA ATACTCTCTA CTTGCAGATG
3961 AACAGCTTAA GGGCTGAGGA CACGGCCGTG TATTACTGTG CGAGAGTGGC CCGGGGGATA
4021 GCAGCTCGAT CGCGAACCAG CTACTTTGAC TACTGGGGCC AGGGAACCCT GGTCACCGTC
4081 TCAAGCGCCT CCACCAAGGG CCCATCGGTC TTCCCGCTAG CACCCTCCTC CAAGAGCACC
4141 TCTGGGGGCA CAGCGGCCCT GGGCTGCCTG GTCAAGGACT ACTTCCCCGA ACCGGTGACG
4201 GTGTCGTGGA ACTCAGGCGC CCTGACCAGC GGCGTCCACA CCTTCCCGGC TGTCCTACAG
4261 TCTAGCGGAC TCTACTCCCT CAGCAGCGTA GTGACCGTGC CCTCTTCTAG CTTGGGCACC
4321 CAGACCTACA TCTGCAACGT GAATCACAAG CCCAGCAACA CCAAGGTGGA CAAGAAAGTT
4381 GAGCCCAAAT CTTGTGCGGC CGCTTCTCCT GCTACTGCTT CCGCTTCTAC TGCCCCGGCT
4441 ACTGCTACCC CTGAGTCTGC TGAAGGCGCC ACTACTGAGA CTCCTACCAC TGAAACTCCT
4501 GCTGAGAGTG CTAGTGGTCC GCCTGCTCCT TCTGAATCCG CCACTGAGGA ATCTGGTGAG
4561 GCTTCTACCA GTAGTACTGC TGAAGAGGGT CCTGCTGAAC CGGGCTCTCC TGCCCCTACT
4621 CCTGCTGCTA CTCCGGCTGA GACCTCCTCT GAACCTCCTG AGGAACCTGG TGGTGCCGGT
4681 ACTCCGGCTG GCACTACTAC CGGTGCTGAG ACTGAATCTG CTACTGAGGG TGGTGCCAGT
4741 AGTGCTCCTG CTTCTCCTAC TGGCGGTGCT CCTTCCTCTG GTGAAACCAC TACTGAGGGT
4801 GGCCCGGCCG GTCCTGCTCC TGCTACTGCT GCCCCTACCG GTGGTGGCGC TGGTGGTGAA

TABLE 20-continued

unannotated DNA sequence of pM160G12:URP12
4861 GGTTCTGCTG GCGGTGGTAC TGGTGAGGAA GGCGGTGGTG GTGCTCCGGA GGGCAGTGGT
4921 GGTGGTCCTG AAGGCCCTAC TCCTGCCACT GAGGCTAGTC CGGAAGGTGC TCCTCCTGGT
4981 TCTACCTCCA CTTCTGGTCC TGGCGAGGCT GCCTCTCCGA CTAGTAGTCC TGGTTAATGA
5041 TAAAACGCGT GATGAGAATT CACTGGCCGT CGTTTTACAA CGTCGTGACT GGGAAAACCC
5101 TGGCGTTACC CAACTTAATC GCCTTGCAGC ACATCCCCCT TTCGCCAGCT GGCGTAATAG
5161 CGAAGAGGCC CGCACCGATC GCCCTTCCCA ACAGTTGCGC AGCCTGAATG GCGAATGGCG
5221 CCTGATGCGG TATTTTCTCC TTACGCATCT GTGCGGTATT TCACACCGCA TACGTCAAAG
5281 CAACCATAGT ACGCGCCCTG TAGCGGCGCA TTAAGCGCGG CGGGTGTGGT GGTTACGCGC
5341 AGCGTGACCG CTACACTTGC CAGCGCCTTA GCGCCCGCTC CTTTCGCTTT CTTCCCTTCC
5401 TTTCTCGCCA CGTTCGCCGG CTTTCCCCGT CAAGCTCTAA ATCGGGGGCT CCCTTTAGGG
5461 TTCCGATTTA GTGCTTTACG GCACCTCGAC CCCAAAAAAC TTGATTTGGG TGATGGTTCA
5521 CGTAGTGGGC CATCGCCCTG ATAGACGGTT TTTCGCCCTT TGACGTTGGA GTCCACGTTC
5581 TTTAATAGTG GACTCTTGTT CCAAACTGGA ACAACACTCA ACTCTATCTC GGGCTATTCT
5641 TTTGATTTAT AAGGGATTTT GCCGATTTCG GTCTATTGGT TAAAAAATGA GCTGATTTAA
5701 CAAAAATTTA ACGCGAATTT TAACAAAATA TTAACGTTTA CAATTTTATG GTGCAGTCTC
5761 AGTACAATCT GCTCTGATGC CGCATAGTTA AGCCAGCCCC GACACCCGCC AACACCCGCT
5821 GACGCGCCCT GACGGGCTTG TCTGCTCCCG GCATCCGCTT ACAGACAAGC TGTGACCGTC
5881 TCCGGGAGCT GCATGTGTCA GAGGTTTTCA CCGTCATCAC CGAAACGCGC GA

TABLE 21

Examples of human Glycine Rich Sequences (GRS) for use in designing human-specific URPs

Accession	Gly (%)	GRS length	Gene length	Hydro-phobics	Predicted Function
NP_000217	62	135	622	Yes	keratin 9
NP_631961	61	73	592	Yes	TBP-associated factor 15 isoform 1
NP_476429	65	70	629	Yes	keratin 3

TABLE 21-continued

Examples of human Glycine Rich Sequences (GRS) for use in designing human-specific URPs

Accession	Gly (%)	GRS length	Gene length	Hydro-phobics	Predicted Function
NP_000418	70	66	316	Yes	loricrin, cell envelope
NP_056932	60	66	638	Yes	cytokeratin 2

TABLE 22

Additional examples of human Glycine Rich Sequences for use in designing human-specific URPs

Accession	Sequences	Number of amino acids
NP_006228	GPGGGGGPGGGGGPGGGGPGGGGGGGPGGGGGGPGG	37
NP_787059	GAGGGGGGGGGGGGGSGGGGGGGGAGAGGAGAG	33
NP_009060	GGGSGSGGAGGGSGGGSGSGGGGGGAGGGGGG	32
NP_031393	GDGGGAGGGGGGGGSGGGGSGGGGGGG	27
NP_005850	GSGSGSGGGGGGGGGGGGSGGGGGG	25
NP_061856	GGGRGGRGGGRGGGGRGGGRGGG	22
NP_787059	GAGGGGGGGGGGGGGSGGGGGGGGAGAGGAGAG	33
NP_009060	GGGSGSGGAGGGSGGGSGSGGGGGGAGGGGGG	32
NP_031393	GDGGGAGGGGGGGGSGGGGSGGGGGGG	27
NP_115818	GSGGSGGSGGGPGPGPGGGGG	21
NP_376532	GEGGGGGGEGGGAGGGSG	18
NP_065104	GGGGGGGGDGGG	12

GGGSGSGGAGGGSGGGSGSGGGGGGAGGGGGGSSGGGSGTAGGHSG
POU domain, class 4, transcription factor 1 [*Homo sapiens*]

TABLE 22-continued

Additional examples of human Glycine Rich Sequences for use in designing human-specific URPs

GPGGGGGPGGGGGPGGGGPGGGGGGGPGGGGGGPGGG
YEATS domain containing 2 [*Homo sapiens*]

GGSGAGGGGGGGGGGGSGSGGGGSTGGGGGTAGGG
AT rich interactive domain 1B (SWI1-like) isoform 3; BRG1-binding protein ELD/OSA1; Eld (eyelid)/Osa protein [*Homo sapiens*]

GAGGGGGGGGGGGGGSGGGGGGGGAGAGGAGAG
AT rich interactive domain 1B (SWI1-like) isoform 2; BRG1-binding protein ELD/OSA1; Eld (eyelid)/Osa protein [*Homo sapiens*]

GAGGGGGGGGGGGGGSGGGGGGGGAGAGGAGAG
AT rich interactive domain 1B (SWI1-like) isoform 1; BRG1-binding protein ELD/OSA1; Eld (eyelid)/Osa protein [*Homo sapiens*]

GAGGGGGGGGGGGGGSGGGGGGGGAGAGGAGAG
purine-rich element binding protein A; purine-rich single-stranded DNA-binding protein alpha; transcriptional activator protein PUR-alpha [*Homo sapiens*]

GHPGSGSGSGGGGGGGGGGGGSGGGGGGAPGG
regulatory factor X1; trans-acting regulatory factor 1; enhancer factor C; MHC class II regulatory factor RFX [*Homo sapiens*]

GGGGSGGGGGGGGGGGGGSGSTGGGGSGAG
bromo domain-containing protein disrupted in leukemia [*Homo sapiens*]

GGRGRGGRGRGSRGRGGGGTRGRGRGRGGRG
unknown protein [*Homo sapiens*]

GSGGSGGSGGGPGPGPGGGGGPSGSGSGPG
PREDICTED: hypothetical protein XP_059256 [*Homo sapiens*]

GGGGGGGGGGGRGGGGRGGGRGGGGEGGG
zinc finger protein 281; ZNP-99 transcription factor [*Homo sapiens*]

GGGGTGSSGGSGSGGGGSGGGGGGGSSG
RNA binding protein (autoantigenic, hnRNP-associated with lethal yellow) short isoform; RNA-binding protein (autoantigenic); RNA- binding protein (autoantigenic, hnRNP-associated with lethal yellow) [*Homo sapiens*]

GDGGGAGGGGGGGGSGGGGSGGGGGGG
signal recognition particle 68 kDa [*Homo sapiens*]

GGGGGGGSGGGGGSGGGGSGGGRGAGG
KIAA0265 protein [*Homo sapiens*]

GGGAAGAGGGGSGAGGGSGGSGGRGTG
engrailed homolog 2; Engrailed-2 [*Homo sapiens*]

GAGGGRGGGAGGEGGASGAEGGGGAGG
RNA binding protein (autoantigenic, hnRNP-associated with lethal yellow) long isoform; RNA-binding protein (autoantigenic); RNA-binding protein (autoantigenic, hnRNP-associated with lethal yellow) [*Homo sapiens*]

GDGGGAGGGGGGGGSGGGGSGGGGGGG
androgen receptor; dihydrotestosterone receptor [*Homo sapiens*]

GGGGGGGGGGGGGGGGGGGGGGGEAG
homeo box D11; homeo box 4F; Hox-4.6, mouse, homolog of; homeobox pro- tein Hox-D 11 [*Homo sapiens*]

GGGGGGSAGGGSSGGGPGGGGGGAGG
frizzled 8; frizzled (Drosophila) homolog 8 [*Homo sapiens*]

GGGGGPGGGGGGGPGGGGGPGGGGG
ocular development-associated gene [*Homo sapiens*]

GRGGAGSGGAGSGAAGGTGSSGGGG
homeo box B3; homeo box 2G; homeobox protein Hox-B3 [*Homo sapiens*]

GGGGGGGGGGGSGGSGGGGGGGGGG
chromosome 2 open reading frame 29 [*Homo sapiens*]

GGSGGGRGGASGPGSGSGGPGGPAG
DKFZP564F0522 protein [*Homo sapiens*]

TABLE 22-continued

Additional examples of human Glycine Rich Sequences for use in designing human-specific URPs
GGHHGDRGGGRGGRGGRGGRGGRAG PREDICTED? similar to Homeobox even-skipped homolog protein 2 (EVX-2) [*Homo sapiens*]
GSRGGGGGGGGGGGGGGGGGAGAGGG ras homolog gene family, member U; Ryu GTPase; Wnt-1 responsive Cdc42 homolog; 2310026M05Rik; GTP-binding protein like 1; CDC42-like GTPase [*Homo sapiens*]
GGRGGRGPGEPGGRGRAGGAEGRG scratch 2 protein; transcriptional repressor scratch 2; scratch (*drosophila* homolog) 2, zinc finger protein [*Homo sapiens*]
GGGGGDAGGSGDAGGAGGRAGRAG nucleolar protein family A, member 1; GAR1 protein [*Homo sapiens*]
GGGRGGRGGGRGGGGRGGGRGGG keratin 1; Keratin-1; cytokeratin 1; hair alpha protein [*Homo sapiens*]
GGSGGGGGGSSGGRGSGGGSSGG hypothetical protein F1131413 [*Homo sapiens*]
GSGPGTGGGGSGSGGGGGGSGGG one cut domain, family member 2; onecut 2 [*Homo sapiens*]
GARGGGSGGGGGGGGGGGGGGPG POU domain, class 3, transcription factor 2 [*Homo sapiens*]
GGGGGGGGGGGGGGGGGGGGGDG PREDICTED: similar to THO complex subunit 4 (Tho4) (RINA and export factor binding protein 1) (REF1-I) (Ally of AML-1 and LEF-1) (Aly/REF) [*Homo sapiens*]
GGTRGGTRGGTRGGDRGRGRGAG PREDICTED: similar to THO complex subunit 4 (Tho4) (RNA and export factor binding protein 1) (REF1-I) (Ally of AML-1 and LEF-1) (Aly/REF) [*Homo sapiens*]
GGTRGGTRGGTRGGDRGRGRGAG POU domain, class 3, transcription factor 3 [*Homo sapiens*]
GAGGGGGGGGGGGGGGAGGGGGG nucleolar protein family A, member 1; GAR1 protein [*Homo sapiens*]
GGGRGGRGGGRGGGGRGGGRGGG fibrillarin; 34-kD nucleolar scleroderma antigen; RNA, U3 small nucleolar interacting protein 1 [*Homo sapiens*]
GRGRGGGGGGGGGGGGRGGGG zinc finger protein 579 [*Homo sapiens*]
GRGRGRGRGRGRGRGRGRGGAG calpain, small subunit 1; calcium-activated neutral proteinase; calpain, small polypeptide; calpain 4, small subunit (30 K); calcium-dependent protease, small subunit [*Homo sapiens*]
GAGGGGGGGGGGGGGGGGGGG keratin 9 [*Homo sapiens*]
GGGSGGGHSGGSGGGHSGGSGG forkhead box D1; forkhead-related activator 4; Forkhead, homolog-like 8; forkhead (*Drosophila*)-like 8 [*Homo sapiens*]
GAGAGGGGGGGGAGGGGSAGSG PREDICTED? similar to RIKEN cDNA C230094B15 [*Homo sapiens*]
GGPGTGSGGGGAGTGGGAGGPG GGGGGGGGGAGGAGGAGSAGGG cadherin 22 precursor; ortholog of rat PB-cadherin [*Homo sapiens*]
GGDGGGSAGGGAGGGSGGGAG AT-binding transcription factor 1; AT motif-binding factor 1 [*Homo sapiens*]
GGGGGGSGGGGGGGGGGGGG eomesodermin; t box, brain, 2; eomesodermin (*Xenopus laevis*) homolog [*Homo sapiens*]
GPGAGAGSGAGGSSGGGGGPG phosphatidylinositol transfer protein, membrane-associated 2; PYK2 N- terminal domain-interacting receptor 3; retinal degeneration B alpha 2 (*Drosophila*) [*Homo sapiens*]

TABLE 22-continued

Additional examples of human Glycine Rich Sequences for use in designing human-specific URPs
GGGGGGGGGGGSSGGGGSSGG sperm associated antigen 8 isoform 2; sperm membrane protein 1 [*Homo sapiens*]
GSGSGPGPGSGPGSGPGHGSG PREDICTED: RNA binding motif protein 27 [*Homo sapiens*]
GPGPGPGPGPGPGPGPGPGPG AP1 gamma subunit binding protein 1 isoform 1; gamma-synergin; adaptor-related protein complex 1 gamma subunit-binding protein 1 [*Homo sapiens*]
GAGSGGGGAAGAGAGSAGGGG AP1 gamma subunit binding protein 1 isoform 2; gamma-synergin; adaptor-related protein complex 1 gamma subunit-binding protein 1 [*Homo sapiens*]
GAGSGGGGAAGAGAGSAGGGG ankyrin repeat and sterile alpha motif domain containing 1; ankyrin repeat and SAM domain containing 1 [*Homo sapiens*]
GGGGGGGSGGGGGGSGGGGGG methyl-CpG binding domain protein 2 isoform 1 [*Homo sapiens*]
GRGRGRGRGRGRGRGRGRGRG triple functional domain (PTPRF interacting) [*Homo sapiens*]
GGGGGGGSGGSGGGGGSGGGG forkhead box D3 *sapiens*
GGEEGGASGGGPGAGSGSAGG sperm associated antigen 8 isoform 1; sperm membrane protein 1 [*Homo sapiens*]
GSGSGPGPGSGPGSGPGHGSG methyl-CpG binding domain protein 2 testis-specific isoform [*Homo sapiens*]
GRGRGRGRGRGRGRGRGRGRG cell death regulator aven; programmed cell death 12 [*Homo sapiens*]
GGGGGGGDGGGRRGRGRGRG regulator of nonsense transcripts 1; delta helicase; up-frameshift mutation 1 homolog (*S. cerevisiae*); nonsense mRNA reducing factor 1; yeast Upflp homolog [*Homo sapiens*]
GGPGGPGGGGAGGPGGAGAG small conductance calcium-activated potassium channel protein 2 isoform a; apamin-sensitive small-conductance Ca2+-activated potassium channel [*Homo sapiens*]
GTGGGGSTGGGGGGGGSGHG SRY (sex determining region Y)-box 1; SRY-related HMG-box gene 1 [*Homo sapiens*]
GPAGAGGGGGGGGGGGGGG transcription factor 20 isoform 2; stromelysin-1 platelet-derived growth factor-responsive element binding protein; stromelysin 1 PDGF- responsive element-binding protein; SPRE-binding protein; nuclear factor SPBP [*Homo sapiens*]
GGTGGSSGSSGSGSGGGRRG transcription factor 20 isoform 1; stromelysin-1 platelet-derived growth factor-responsive element binding protein; stromelysin 1 PDGF- responsive element-binding protein; SPRE-binding protein; nuclear factor SPBP [*Homo sapiens*]
GGTGGSSGSSGSGSGGGRRG Ras-interacting protein 1 [*Homo sapiens*]
GSGTGTTGSSGAGGPGTPGG BMP-2 inducible kinase isoform b [*Homo sapiens*]
GGSGGGAAGGGAGGAGAGAG BMP-2 inducible kinase isoform a [*Homo sapiens*]
GGSGGGAAGGGAGGAGAGAG forkhead box Cl; forkhead-related activator 3; Forkhead, drosophila, homolog-like 7; forkhead (*Drosophila*)-like 7; iridogoniodysgenesis type 1 [*Homo sapiens*]
GSSGGGGGGAGAAGGAGGAG splicing factor p54; arginine-rich 54 kDa nuclear protein [*Homo sapiens*]
GPGPSGGPGGGGGGGGGGGG v-maf musculoaponeurotic fibrosarcoma oncogene homolog; Avian musculoaponeurotic

TABLE 22-continued

Additional examples of human Glycine Rich Sequences for use in designing human-specific URPs
fibrosarcoma (MAF) protooncogene; v-maf musculo- aponeurotic fibrosarcoma (avian) oncogene homolog [*Homo sapiens*]
GGGGGGGGGGGGGGAAGAGG
small nuclear ribonucleoprotein D1 polypeptide 16 kDa; snRNP core protein D1; Sm-D autoantigen; small nuclear ribonucleoprotein D1 polypeptide (16 kD) [*Homo sapiens*]
GRGRGRGRGRGRGRGRGRGG
hypothetical protein H41 [*Homo sapiens*]
GSAGGSSGAAGAAGGGAGAG

SEQUENCE LISTING

The patent contains a lengthy "Sequence Listing" section. A copy of the "Sequence Listing" is available in electronic form from the USPTO web site (http://seqdata.uspto.gov/?pageRequest=docDetail&DocID=US09266964B2). An electronic copy of the "Sequence Listing" will also be available from the USPTO upon request and payment of the fee set forth in 37 CFR 1.19(b)(3).

We claim:

1. A method of treating a plasma kallikrein associated disorder in a subject, the method comprising: administering to the subject an antibody that binds to the active form of human plasma kallikrein; wherein the antibody comprises:

(i) a heavy chain variable region comprising a complementary determining region (CDR) 1 set forth as HYIMM (SEQ ID NO: 166), a CDR2 set forth as GIYSSGGITVYADSVKG (SEQ ID NO: 167), and a CDR3 set forth as RRIGVPRRDEFDI (SEQ ID NO: 1171); and

(ii) a light chain variable region comprising a CDR1 set forth as RASQSISSWLA (SEQ ID NO: 1172), a CDR2 set forth as KASTLES (SEQ ID NO: 1173), and a CDR3 set forth as QOYNTYWT (SEQ ID NO: 1174); wherein the plasma kallikrein associated disorder is hereditary angioedema.

2. The method of claim **1**, wherein the heavy chain variable region of the antibody comprises the amino acid sequence of EVQLLESGGGLVQPGGSLRLSCAASGFTFSHYIMMWVRQAPGKGLEWVSGIYSSGGITVYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAYRRIGVPRRDEFDIWGQGTMVTV SS (SEQ ID NO: 2410), and the light chain variable region of the antibody comprises the amino acid sequence of DIQMTQSPSTLSASVGDRVTITCRASQSISSWLAWYQQKPGKAPKLLIYKASTLESGVPSRF SGSGSGTEFTLTISSLQPDDFATYYCQQYNTYWTFGQGTKVEIK (SEQ ID NO: 2394).

3. The method of claim **1**, wherein the heavy chain variable region of the antibody comprises the amino acid sequence of EVQLLESGGGLVQPGGSLRLSCAASGFTFSHYIMMWVRQAPGKGLEWVSGIYSSGGI TVY ADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAYRRIGVPRRDEFDIWGQGTM VTV SS (SEQ ID NO: 2410), and the light chain variable region of the antibody comprises the amino acid sequence of DIQMTQSPSTLSASVGDRVTITCRASQSISSWLAWYQQKPGKAPKLLIYKASTLESGVP SRF SGSGSGTEFTLTISSLQPDDFATYYCQQYNTYWTFGQGTKVEI (SEQ ID NO: 2395).

4. The method of claim **1**, wherein the antibody is a full-length antibody.

5. The method of claim **4**, wherein the antibody is an IgG molecule.

6. The method of claim **1**, wherein the antibody is an antigen-binding fragment of a full-length antibody.

* * * * *